University of Alberta

Detection of Protozoa in the Saddle Lake Drainage Basin

by

Lisbeth O. Michener ©

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
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Daniel W. Smith

Dr. Daniel W. Smith

Dr. Miodrag Belosevic

Ian Buchanan

Dr. Ian Buchanan

December 21, 2001

Dedicated to

My parents,

Anthony and Wendy Michener,

For their support,

encouragement and

infinite patience.

Abstract

This study involved developing a waterborne protozoa detection procedure while assessing occurrence, potential infectivity and possible sources of *Cryptosporidium parvum* and *Giardia lamblia* within the drainage basin of Saddle Lake, located on the Saddle Lake First Nation Reserve in Alberta, Canada.

Giardia spp. and *Cryptosporidium* spp. were present within the Saddle Lake drainage basin, treatment plant intake and treated water. No samples resulted in cryptosporidiosis in mice while two potable water samples produced giardiasis in gerbils. No correlations were found between the presence of (oo)cysts and total and faecal coliform levels, heterotrophic plate counts, turbidity and pH. Survey results from Saddle Lake First Nation Reserve respondents indicated that low numbers of livestock were present on the reserve in contrast to the surrounding counties.

Recovery trials indicated that this study's detection technique should be further optimized as the method gave rise to low percent recoveries with large amounts of variation.

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List of Symbols and Abbreviations

AE	Alberta Environment
μL	Microlitre
μm	Micron
BSA	Bovine serum albumin
C. *parvum*	*Cryptosporidium parvum*
CsCl	Cesium chloride
DAPI	4',6-diamidonio-2-phenylindole stain
DIC	Differential interference contrast
FA	Immunofluorescence assay (IFA)
FACS	Fluorescent activated cell sorting
FCS	Forward scattered light
FITC	Fluorescein isothiocyanate
FL1	Fluorescent light
G. *lamblia*	*Giardia lamblia*
HPC	Heterotrophic plate count
hr	Hour
ICR	Information collection rule
IFA	Immunofluorescence assay (FA)
Ig/month	Imperial gallons per month
Igpd	Imperial gallons per day
Igpm	Imperial gallons per minute
IMS	Immunomagnetic separation
L	Litre
L/d	Litres per day
log	Logarithm
MAC	Maximum allowable concentration
Min	Minute
mL	Millilitre
mmHg	Centimetre of mercury
NTU	Nephelometric Units
^{o}C	Degree Celsius
(oo)cysts	Both oocysts and cysts
PBST	Phosphate buffered saline solution with 0.01% Tween20
pH	Negative logarithm of the effective hydrogen ion concentration
PI	Propidium iodide
R1, R2, R3	FACS sorting gate regions
rpm	Revolutions per minute
spp.	Species
SSC	Side scattered light
USEPA	United States Environmental Protection Agency
x g	Gravitational force

Chapter 1

1 INTRODUCTION

1.1 PROJECT OVERVIEW

Cryptosporidium parvum and *Giardia lamblia* are two pathogenic protozoa that are of human health concern worldwide due to their virulence, widespread occurrence and resilience to microbial reduction methods and environmental conditions. Initiated by concerned residents of Saddle Lake First Nation community, this project included an examination of the Saddle Lake drainage basin and treated water by novel protozoa detection and infectivity methods and a Saddle Lake resident survey.

This project therefore consisted of three components:

1. The development of a waterborne protozoa detection method and animal infectivity assay.
2. The examination of the watershed and water treatment for the community of Saddle Lake First Nation in northeastern Alberta. Raw and treated water samples were collected from several locations within the Saddle Lake drainage basin and subsequently examined for protozoa occurrence via flow cytometry and their potential for human infectivity through animal infectivity assays.
3. The analysis of a survey conducted by Health Canada of the residents of Saddle Lake First Nation reserve. The objective of the survey was to examine potential sources of *Giardia* spp. and *Cryptosporidium* spp. and to study the potential for significant contamination of the water supply with respect to the two organisms.

1.1.1 Protozoa Detection and Animal Infectivity Assays

Two human pathogenic protozoa, *Cryptosporidium parvum (C. parvum)* and *Giardia lamblia (G. lamblia)*, are commonly found in natural and sometimes treated waters. These species cause two forms of gastroenteritis, cryptosporidiosis and giardiasis, in humans and other animals. They are of particular concern to microorganism reduction efforts in the water industry due to their relatively high infectivity and their resistance to chemical microbial reduction processes (Teunis et al. 1997). Methods of detecting these protozoa have largely offered variable and low recoveries (Clancy et al. 1997b, Bukhari et al. 1998a, Young and Komisar 1999).

This study's methodology used a variation of the United States Environmental Protection Agency's (USEPA) "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/ IMS/ FA (USEPA 1999b). The method consisted of filtering 50 L of raw and at least 1000 L of treated water, eluting the filter, and analyzing a portion of the eluate via animal infectivity and analyzing the remainder of the eluate, after immunomagnetic separation (IMS), by fluorescent activated cell sorting (FACS)[1] and finally by microscopy.

Animal infectivity provides the only irrefutable means of assessing if (oo)cysts[2] are infectious. In this study gerbils and mice were used to detect giardiasis and cryptosporidiosis, respectively. Animal infectivity, however, is an expensive and difficult assay, and as such, is not generally available to water industry laboratories (Neumann et al. 2000b). Therefore an (oo)cyst detection assay using fluorescein isothiocyanate (FITC) staining in conjunction with FACS was developed in this study.

FACS increases the sensitivity of (oo)cyst detection as, unlike straight microscopy, it enables an entire water sample concentrate to be analyzed (Hoffman et al. 1997, Belosevic et al. 2000). With a fluorescent activated cell sorter, particles with certain programmed characteristics can be counted and shunted to a membrane which, in turn, can be assessed by microscopy to visually confirm the presence of (oo)cysts.

1.1.2 Drainage Basin Study

Saddle Lake is located on the Saddle Lake First Nation Reserve, which is approximately 209 kilometres northeast of Edmonton, Alberta, and 22 kilometres west of St. Paul. Approximately 5,352 residents populate the reserve (Robb 2000). Saddle Lake is the raw water source for the water treatment plant, which supplies water to the majority of the residents of the community.

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[1] Note that throughout this thesis, flow cytometry will refer to the particle counts given by the flow cytometer whereas fluorescent activated cell sorter (FACS) will refer to the sorting mechanism component of the machine.

[2] The abbreviation "(oo)cyst" refers to both *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts.

To determine if the population of Saddle Lake was at risk of contracting cryptosporidiosis and giardiasis, water samples from treated and raw water sources throughout the drainage basin were assessed for protozoa and their potential for infectivity.

Also, results from Health Canada's " Land-use, Water and Waste Survey Form" (Appendix A) were compiled and assessed to determine potential sources of protozoa within the drainage basin as they relate to watershed protozoa detections. Survey analysis focused primarily on property land-use characteristics, the number of people and animals residing on properties, manure management practices, and methods of sewage disposal.

1.2 RESEARCH OBJECTIVES

The four goals of this study were:

1. To develop a method for *C. parvum* and *G. lamblia* detection with consistent and good percent recoveries of (oo)cysts;
2. To determine whether *C. parvum* and *G. lamblia* were present in the Saddle Lake drainage basin and treated water;
3. If present, to determine whether the protozoa were infectious; and
4. To examine relationships between land-use or sewage disposal, and the prevalence of *C. parvum* and *G. lamblia*.

1.3 DOCUMENT OUTLINE

This document is organized into seven chapters; introduction, protozoa background, methodology, recovery trials, Saddle Lake drainage basin water quality, Saddle Lake survey and project summary followed by literature cited, Appendix A: Health Canada's "Land-Use, Water and Waste Survey Form" and Appendix B: Land Use Survey Results. The chapters on recovery trials, Saddle Lake drainage basin water quality and Saddle Lake survey are each comprised of an introduction, background information, experimental design, results and discussion subsections. The project summary segment draws conclusions from the study and recommendations for further research.

Chapter 2

2 PROTOZOA BACKGROUND

2.1 HISTORY

2.1.1 C. parvum

The first species of *Cryptosporidium* to be recognized and named was *C. muris* in 1907 by Ernest Edward Tyzzer. In 1910 Tyzzer described the protozoan life cycle when observing that one phase of its life cycle, the environmentally resilient oocyst, was excreted in the faeces of its host (Fayer 1997).

A report revealing 21 cases of diarrhoea due to *C. parvum* infection in conjunction with Acquired Immune Deficiency Syndrome (AIDS) (Anonymous 1982) instilled public interest in cryptosporidiosis. Current (1983) later concluded that the disease might cause self-limiting illness in immunocompetent individuals, thus further inducing interest that resulted in international research on the disease and parasite. Since 1982, cryptosporidiosis cases in both immunocompromised and immunocompetent individuals, often during outbreaks, have been reported worldwide.

2.1.2 G. lamblia

Antony van Leeuwenhok may have discovered *Giardia lamblia* in as early as 1681, however Clifford Dobell described the species as a parasitic protozoan in 1920 (Feely et al. 1990). In 1966 *G. lamblia* was surmised to be a causative agent of waterborne gastrointestinal disease the United States (Fayer 1997).

The species infectious to humans was named *Giardia lamblia* by Kofoid and Christiansen in 1915 and later termed *Giardia duodenalis* by Filice in 1952 (Feely et al. 1990). Although both terms are currently in use, for clarity *Giardia lamblia* or simply *G. lamblia* will be utilized throughout this thesis.

2.2 BIOLOGY AND EPIDEMIOLOGY

2.2.1 C. parvum

Cryptosporidium parvum (Phylum Apicomplexa, Class Sporozoasida, Subclass coccodiasina, Order Eucoccidiorida, Suborder Eimeriorina, Family Cryptosporidiidae) is a coccidian. There are at least four recognized species of *Cryptosporidium* spp. based on morphological and cross-infection analyses. However, only C. *parvum* appears to be of concern to human health, and to 78 other species of mammals (O'Donoghue 1995, Smith et al. 1995).

Cryptosporidium spp. infections have been reported in an estimated 50 countries in over 170 host species. Most infections have likely been transmitted by ingestion of food or water, a few by inhalation and others through direct host-host contact (O'Donoghue 1995).

C. *parvum* is transmitted via the faecal-oral route in the infectious phase of the environmentally resilient oocyst. Oocysts, which are spherical and between 3 to 6 μm in diameter (Tzipori 1988), can be transmitted through food, water or close contact with infected animals or humans (Fayer 1997). Oocysts are the result of asexual and sexual reproduction within the cells of the microvillus within the small intestine. Oocysts release sporozoites that attach to microvilli and become trophozoites. Trophozoites then develop into type I and then II meronts, which release merozoites, which form micro- and macrogamonts, which then form thin or thick walled oocysts (Fayer 1997). Excystation is enhanced by reducing conditions such as the presence of bile salts and pancreatic enzymes (Fayer 1997) although carbon dioxide and temperature can also influence the process (O'Donoghue 1995).

2.2.2 G. lamblia

According to the Society of Protozoologist committee, the genus *Giardia* belongs to the subphylum Sarcomastigophora, superclass Mastigophora, class Zoomastigophorea, order Diplomonadida, and family Hexamitidae (Meyer 1990).

Giardia lamblia forms cysts that protect the organism from environmental stresses such as low nutrient levels, temperature and pH fluctuations so that they may survive for an extended period of time in a harsh environment. Cysts are elliptical and range from 6 to 10 μm in length (Feely et al. 1990).

The life cycle of G. *lamblia* is less complex than that of C. *parvum.* The parasitic protozoan exists in two morphological forms: the flagellated trophozoite, which reproduce asexually, and the cyst, which, like the oocyst of C. *parvum*, is environmentally resistant. Cysts are excreted in the faeces and are infective after the cyst has excysted, releasing their two trophozoites into the intestine of the new host. Trophozoites are approximately 12 to 15 μm long and 5 to 9 μm wide and are pear-shaped (Feely et al. 1990). Trophozoites attach to the outer surface of the small intestine where they multiply asexually. As the trophozoites pass through the intestine they encyst and are released in the faeces as cysts (Smith et al. 1995).

2.3 SYMPTOMS AND IMMUNITY

2.3.1 C. parvum

The most common symptom of cryptosporidiosis is watery stool. Diarrhoea may contain mucus but rarely blood or leucocytes. Weight loss may occur due to increased numbers of bowel movements - up to10 times a day. Other manifestations of cryptosporidiosis include abdominal pain (cramps), fever, nausea, malaise, weakness or fatigue, headache, myalgia, and vomiting (O'Donoghue 1995) and infections may spread or develop in the small intestine, oesophagus, stomach, appendix, colon and rectum, creating other relevant symptoms (O'Donoghue 1995).

Small intestinal histology during C. *parvum* infections reveals varying levels of atrophy and larger crypt size. Should the respiratory epithelium become infected, it may show similar symptoms in conjunction with deciliation, hyperplasty or hypertrophy (O'Donoghue 1995).

The period between infection and oocyst excretion (prepatent period) generally ranges from 2 to 14 days and the period of oocyst excretion (patent period) may last from days to months. The length and magnitude of infection depends not only on the virulence of the C. *parvum* strain, but also on the state of the host's immune system although there are reports of varied manifestations occurring in individuals with ranging immune-system competencies. Individuals with competent immune systems may experience symptoms for one to two weeks. In contrast, individuals with immunocompromised immune systems may experience chronic or even life-threatening infections of varying severity (O'Donoghue 1995).

Generally young animals and children are more prone to *Cryptosporidium* spp. infections (O'Donoghue 1995). Children younger than five seem to be more susceptible to cryptosporidiosis as are malnourished, pregnant, and concurrently infected individuals (Mann et al. 1986, Public Health Laboratory Service Study Group 1990).

Drugs that may weaken the immune system and increase an individual's susceptibility to infection include immunosuppressive drug therapy, steroids, or chemotherapeutic agents. Those with congenital, acquired lymphocyte or gammaglobulin immunodeficiencies (compromised immune systems), leukemia, lymphoma or other cancers, haematological malignancy, T cell depletion prior to bone marrow transplantation, acquired immune deficiency syndrome (AIDS), or those undergoing post organ or tissue transplantation may also be predisposed to cryptosporidiosis (Petersen 1992, O'Donoghue 1995).

Cryptosporidiosis accounts for 10 to 15 percent of chronic diarrhoea in individuals with HIV in the USA and 30 to 50 percent in developing countries (Colebunders et al. 1987, Laughon et al. 1988).

Infections in individuals with more competent immune systems can create an immunity to further infection. Immunological mechanisms that act against *Cryptosporidium* spp. were summarized by Zu (1992) and O'Donoghue (1995).

2.3.2 G. lamblia

Giardiasis can manifest itself with flatulence, sulphurous belching, abdominal cramps, and/ or distension. Initial watery diarrhoea occurs frequently and later becomes "bulky, sometimes frothy, greasy and offensive" (Smith et al. 1995). Should the disease develop more chronically, weight loss, malaise, and malabsorption of vitamins A and B_{12} and D-xylose may occur (Smith et al. 1995).

As with cryptosporidiosis, giardiasis may occur asymptomatically in an estimated 30 to 70 percent of *Giardia* spp. infections (Smith et al. 1995). Whether or not an individual is symptomatic or asymptomatic, *Giardia* spp. infections appear to depend on three factors: local intestinal conditions, differences in pathogenicity of different strains of the parasite, and host immune response (Janoff and Smith 1990). When episodically exposed to the parasite, populations are likely to display symptoms. However, those with recurrent or chronic exposures to *Giardia* spp. are often asymptomatic. In developing countries, *Giardia lamblia* is an important cause of disease in children. Where endemic, giardiasis is more prevalent in children less than 10 and decreases with age; thus indicating the importance of acquired immune responses (Islam 1990, Janoff and Smith 1990).

Like cryptosporidiosis, giardiasis is self-limiting in most individuals. On average, the giardiasis prepatent period is 9.1 days and the incubation period is generally 1 to 2 weeks. Up to 107 cysts may be excreted per gram of faeces although the period of cyst excretion varies (Smith et al. 1995).

2.4 DIAGNOSIS

2.4.1 C. parvum

Diagnosis of cryptosporidiosis is generally through direct microscopic examination of faeces for *Cryptosporidium* spp. oocysts (O'Donoghue 1995). Oocyst excretion often occurs during the manifestations of clinical symptoms of cryptosporidiosis in both the immunocompetent and immunocompromised hosts. Oocysts are released from infected humans in their faeces at the rate of approximately one million oocysts per day (Daniel et al. 1996). Oocysts may be excreted after the clinical symptoms subside. Should infections other than intestinal occur, oocysts may also be found in bile, sputum, or respiratory aspirates. Expertise is required in identifying stained oocysts due to the variability of oocyst characteristics and stain uptake; which is likely linked with their age and viability (O'Donoghue 1995).

Other indirect diagnostic methods such as comparative symptomatology or clinical parameters do not seem to be satisfactory (O'Donoghue 1995) although prior exposure to C. *parvum* may be assessed through immunoserological tests for the detection of specific host antibodies against the parasite (O'Donoghue 1995).

2.4.2 G. lamblia

The diagnosis of *Giardia* spp. infections is generally by microscopic examination of faecal samples. However this method may not be sufficient when examining low levels of excreted cysts. As such, detection assay methods have progressed to include serology and immunoassays (Smith et al. 1995). The detection of *Giardia* spp. antigens in stool reflects current infection whereas anti-*Giardia* spp. antibodies indicate present or past giardiasis infections (Wolfe 1990).

2.5 TREATMENT

2.5.1 C. parvum

Anti-parasitic and anti-microbial drug treatments have not proven effective for cryptosporidiosis to date. Immunocompetent patients should receive oral or intravenous hydration and electrolyte replacements (O'Donoghue 1995). In individuals with compromised immune systems, cryptosporidiosis may be life threatening; therefore clinical trials for chemotherapy treatment are ongoing (Marshall et al. 1997). For a comprehensive summary of previous clinical research into the treatment of cryptosporidiosis see O'Donoghue (1995).

2.5.2 G. lamblia

Unlike cryptosporidiosis, giardiasis may be treated with many drugs including metronidazole, nitroimidazole compounds, quinacrine and furazolidone. Rehydration therapy is recommended should the disease cause severe dehydration (Smith et al. 1995).

Chapter 3

3 METHODOLOGY

3.1 WATERBORNE PROTOZOA DETECTION AND INFECTIVITY ASSAYS

The following experimental protocol parallels the United States Environmental Protection Agency's (USEPA) "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/ IMS/ FA" (USEPA 1999b), with several variations. See Figure 1 for a procedural flow diagram.

3.1.1 Sampling

3.1.1.1 Treated Water

At least 1,000 L of drinking water was passed through Genera Technologies Filta-max™ filter[3] at a flow rate between 2 and 4 L/min. The apparatus consisted of: 0.5 m Tygon formula R-3603 tubing attached to a faucet followed by Genera Technology's[4] Filta-Max housing and filter, a small section of Tygon tubing, an ABB water metre and a garden hose to drain the system. At least 100 L of source water was run through the system (minus the filter) prior to each filtration.

Filter housings were plugged, placed in Zip Loc plastic bags and transported in coolers. They were then stored at 4°C until elution processing.

3.1.1.2 Raw Water

50 L of raw water was passed through a Filta-Max™ filter at a flow rate between 2 and 4 L/min. The sampling apparatus consisted of: at least 15 m Tygon formula R-3603 tubing, a Simer® Model 2825ss portable utility pump, Filta-max™ housing and filter, a short piece of Tygon tubing connected to an ABB water metre, and finally a garden hose to drain the system. At least 100 L of source water was run through the system (minus the filter) prior to each filtration.

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[3] Filta-Max has been validated for 50 L raw water sample volumes under the USEPA performance based measurements system (Bukhari 2000).

[4] IDEXX Genera, Lynx Business Park, Fordham Road Newmarket, Cambridgeshire, CB8 7NY, www.genera.co.uk

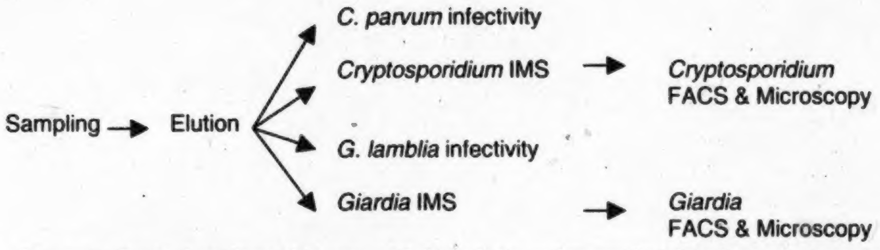
Filter housings were plugged, placed in Zip Loc plastic bags and transported in coolers. They were then stored at 4°C until elution processing.

3.1.2 Elution

Filters were eluted according to the methods outlined in Bukhari (2000) and Filta-Max™ Protocol manual.

A 3 μm cellulose nitrate membrane was placed on the concentrator column base under 600 mL phosphate buffered saline solution with 0.01% Tween®20 (PBST). The screw holding the filter's compressed foam pads was screwed onto the plunger head. The elution column was assembled and the screw was released by an Allen key, allowing the foam pads to decompress and expand. The foam pads were then gently plunged up and down in PBST 20 times. The concentrator column was removed from the plunger manifold, the plunger was pumped to empty the stainless steel tube and the concentrator column was placed on a magnetic stir plate. The eluate was drained through the membrane filter with a hand pump and a maximum pressure of 300 mm Hg until approximately 20 mL of the eluate remained. The excess solution was poured off into a beaker and the process was repeated with only 10 plunges.

When either the membrane filter clogged or when the elution process was complete, the membrane(s) was washed by hand by kneading twice in a zip-loc bag with 10 mL PBST for 1 minute per washing. The sample was pipetted into the beaker that contained the excess eluate. Each sample was then divided into four equal portions into 15 mL centrifuge tubes for further processing: *C. parvum* infectivity, *G. lamblia* infectivity, *C. parvum* flow cytometry and *G. lamblia* flow cytometry (Figure 1). The sub-samples were stored at 4°C until further processing.



• Figure 1: Procedural flow diagram outlining eluate sub-sampling into four sub-samples for four different analyses.

3.1.3 *C. parvum* Infectivity Assay

3.1.3.1 Inoculation

C. parvum infectivity methodology was adapted from Belosevic et al. (2000).

Sub-samples were centrifuged at 1553 x g for 15 minutes to reduce the volume to 1 mL. If too thick, the sample volume was further reduced and the sample was diluted. It was then equally divided to orally inoculate 10 five-day-old neonatal CD-1 mice. The mice were removed from their mothers one hour prior to inoculation

The inoculations were performed in 1 mL polypropylene syringe attached to a feeding needle. 50 μL was dispensed into each stomach. Ten mice were inoculated per subsample.

The neonatal mice were then placed into a cage with a lactating mother. After 7 days the mice were assessed for *C. parvum* infection.

3.1.3.2 Detection

Mice were sacrificed by cervical dislocation. The large intestine was removed and placed in a 15 mL polypropylene test tube containing 10 mL deionised water. The intestine was homogenized for 45 to 60 seconds at maximum power by a Sorvall Omni Mixer equipped with an aerosol containment unit.

The homogenate was dispensed into a 15 mL polypropylene tube and washed by centrifugation at 2000 x g for 15 minutes. The supernatant was removed and the pellet re-suspended in 10 mL deionised water with 0.01% Tween 20. The suspensions were washed again and the supernatant was once again discarded.

20 µL of the pellet was removed and placed into a 6 mL polystyrene flow cytometer test tube attached to a 35-µm sieve. The sieve was flushed with 400 µL 1% bovine serum albumin (BSA) in phosphate buffered saline solution (PBS). The suspension was incubated for at least 15 minutes to block the non-specific absorption of the monoclonal antibody in the proceeding step.

Following the blocking, 100 µL of a 1:400 dilution of fluorescein-labelled anti-*C. parvum* oocyst monoclonal antibody (manufactured by ImmuCell) was added. The antibody was diluted in 1% bovine serum albumin phosphate buffered saline solution (BSA/ PBS) and incubated at 37°C for 30 minutes.

The suspensions were analysed using Becton-Dickinson's CellQuest™ application for flow cytometry according to the parameters outlined in Belosevic et al. (2000). Those intestinal homogenates that scored greater than 1.25 were analysed by microscopy to verify the presence or absence of *C. parvum* oocysts.

3.1.4 *G. lamblia* Infectivity Assay

3.1.4.1 Inoculation

Sub-samples were spun at 1013 x g for 10 minutes to reduce the suspension volume to 1 mL.[6] If the resultant suspension was too thick it was reduced in volume and diluted to orally inoculate 4 Mongolian gerbils with equal portions of the sample.

3.1.4.2 Cyst Detection

The methodology for cyst detection in Mongolian gerbil faeces was adapted from Belosevic et al. (1983).

Faeces were collected for three sequential days between 10 and 20 days (post inoculation) prior to examination of intestines.

The Mongolian gerbils were placed on false-bottom cages, according to sample number and earmarks, with a small amount of water in the cages to soften the faeces. 1.5 mL MilliQ water was dispensed into 14 mL centrifuge tubes and tubes were then weighed. After approximately 1 hour the faeces were collected in corresponding tubes and the tubes were re-weighed.

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[6] Note that no vortexing was performed with *Giardia* spp. cysts due to their fragile structure.

The contents of each tube was emulsified and gently pipetted onto 3 mL of 1 M sucrose in glass culture tubes. Culture tubes were then centrifuged for 15 minutes at 285 x g. Following centrifugation, the *G. lamblia* layer was directly above the sucrose layer; the top layer was therefore removed and discarded by pipette and the subsequent "milky" layer was pipetted onto 10 μm filters resting on 15 mL centrifuge tubes. These tubes were toped with MilliQ water and centrifuged for 10 minutes at 388 x g.

The suspension was concentrated to 1 mL and the pellet was re-suspended by gently pipetting the solution up and down. Four haemocytometer counts per gerbil were performed.

3.1.4.3 Trophozoite Detection

The methodology for trophozoite detection in Mongolian gerbil intestines was adapted from Belosevic et al. (1983).

The Mongolian gerbils were terminated by cervical dislocation. The entire small intestine was removed and sectioned into four pieces: section 1 being closest to the stomach and 4 being closest to the caecum. Each segment was placed in 3 mL PBS within a 15 mL centrifuge tube and the tubes were placed on ice for 30 minutes.

The intestinal segments were cut longitudinally and the mucosal layer was scraped off. All of the remains from one intestinal segment were placed into the centrifuge tube prior to 30 minutes on ice. The suspension in the tube was vortexed and the contents were strained through gauze into another 15 mL centrifuge tube containing 3 mL PBS. The tube was toped up with PBS and centrifuged for 15 minutes at 285 x g.

The volume was reduced to 1 mL and two haemocytometer counts were performed on the suspensions resulting from segments 1 and 2 and one count was performed on fragments 3 and 4.

3.1.5 Detection Analysis

3.1.5.1 Cryptosporidium spp. IMS

Cryptosporidium spp. flow cytometry sub-samples underwent immunomagnetic separation (IMS) using Miltenyi Biotech[7] MiniMACS columns and MicroBeads. Sub-samples were spun in 15 mL centrifuge tubes for 15 minutes at 1553 x g. The supernatant was aspirated down to 0.5 mL and the pellet was re-suspended in 9.5 mL IMS buffer[8] and incubated for half an hour at room temperature. Ten-µL anti-*Cryptosporidium* Antibody (ImmuCell) was added. The suspension was incubated for 30 minutes at room temperature then spun at 1553 x g for 15 minutes. The volume was reduced to 0.5 mL, 20-µL MicroBead solution was added and the suspension was re-suspended. The sample was incubated at 4°C for 20 minutes.

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[7] Miltenyi Biotech Inc., www.miltenyibiotec.com

After placement on the MiniMACS magnet, the MACS Separation Column and Pre-Separation Column were rinsed with 500-µL IMS buffer. The suspension was pipetted into the pre-separation column and the centrifuge tube was rinsed three times with 500-µL IMS buffer. The pre-separation column was rinsed with 500-µL IMS buffer and the MiniMACS separation column was rinsed twice with 500-µL IMS buffer. The MACS separation column was then removed from the magnet and flushed 5 times with the plunger using 500-µL IMS buffer per flush.

The resultant positive fraction was then centrifuged at 1553 x g for 15 minutes, reduced to 0.5 to 1 mL, and stored at 4°C until flow cytometer and FACS analysis.

3.1.5.2 Cryptosporidium spp. FACS

Water samples of 0.5 to1.0 mL, labelled with FITC-conjugated antibody were stored in the dark at 4°C until flow cytometer (FACS*Calibur*, Serial number E1444, Becton-Dickinson Immunocytometry Systems, 2350 Qume Drive, San Jose, Ca) examination. The following instrument settings were used:

1. Forward scatter photodiode setting = E00 with 4.00 amp gain, linear mode;
2. Side scatter photomultiplier setting = 402 with 4.00 amp gain, linear mode;
3. FL1 photomultiplier setting = 470 with 1.00 amp gain, log mode;
4. FL2 photomultiplier setting = 578 with 1.00 amp gain, log mode;
5. Threshold primary parameter = FSC at a value of 52; and

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[8] Phosphate buffered saline with 2mM EDTA and 0.5% bovine serum albumin with a pH of 7.2.

6. Compensation = FL1 - 1.3% FL2.

A standard sample data acquisition template was used, based on preliminary studies with known *Cryptosporidium* spp. -positive samples to determine the range of FSC, SSC and FL1 values that a particle would need to fall within to be considered as a possible *Cryptosporidium* spp. oocyst. Region R1 (on a FSC vs. SSC plot) defined an area within which particles with a size and internal complexity consistent with *Cryptosporidium* spp. would be located. Region R2 (on a FSC vs. FL1 plot) defined an area within which particles of the size and fluorescence of labelled *Cryptosporidium* spp. oocysts would be located.

A portion of each sample was run at the HI flow rate setting for 2 minutes to collect a data file for each sample. The remainder of each sample was run at the LOW flow rate setting, and any particles falling within both regions, R1 and R2, were sorted out. Sorted samples were passed through a transparent cell culture insert (12 well format, 1-μm pore size, Becton Dickinson, Franklin Lakes, NJ) to retain oocysts and other particles. Following sorting, the membrane was cut out of the holder and placed on a glass slide. Seven-μL water was added to the membrane, and a cover glass placed on top. The cover glass was ringed with nail polish. The membrane was examined using an epifluorescence microscope, with viewing at 400x magnification, and any *Cryptosporidium* spp. oocysts were identified and counted.

3.1.5.3 *Giardia spp. IMS*

Giardia spp. sub-samples were spun at 1013 x g for 10 minutes and the volume was reduced to 0.5 mL. The cysts were re-suspended in 5 mL PBS and 5% calf serum. The suspension was incubated for 15 minutes at room temperature. One mL anti-*G. lamblia* monoclonal antibody hybridoma supernatant[9] was added and the suspension was incubated for 30 minutes at 37°C. Two-μL anti-mouse Ig polyclonal (Pharmigen) was added followed by an incubation period of 30 minutes at 37°C. The suspension was then spun at 1013 x g for 10 minutes and aspirated down to 0.5 mL. Finally 20-μL of MicroBeads was added and the solution was incubated at 4°C for at least 20 minutes.

Following placement on the MiniMACS magnet, the MiniMACS Separation Column and Pre-Separation Column were rinsed with 500-μL IMS buffer. The suspension was pipetted into the pre-separation column and centrifuge tube was rinsed three times with 500-μL IMS buffer. The pre-separation column was then rinsed with 500-μL IMS buffer and the MiniMACS separation column was rinsed twice with 500-μL IMS buffer. The separation column was then removed from the magnet and flushed five times with the plunger using 500-μL IMS buffer per flush.

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[9] Kind gift of Dr. G. Faubert.

The resultant positive fraction was then spun at 1013 x g for 10 minutes, reduced to 0.5 to 1 mL, and stored at 4°C until flow cytometry and FACS analyses.

3.1.5.4 Giardia spp. FACS

Water samples of 0.5 to1.0 mL, labelled with FITC-conjugated antibody were stored in the dark at 4°C until examination in a flow cytometer (FACS*Calibur*, Serial number E1444, Becton-Dickinson Immunocytometry Systems, 2350 Qume Drive, San Jose, Ca). The following instrument settings were used:

1. Forward scatter photodiode setting = E-01 with 7.04 amp gain, linear mode;
2. Side scatter photomultiplier setting = 350 with 4.00 amp gain, linear mode;
3. FL1 photomultiplier setting = 480 with 1.00 amp gain, log mode;
4. FL2 photomultiplier setting = 578 with 1.00 amp gain, log mode;
5. Threshold primary parameter = FSC at a value of 52; and
6. Compensation = FL1 - 1.3% FL2.

A standard sample data acquisition template was used, based on preliminary studies with known *Giardia* spp.-positive samples to determine the range of FSC, SSC and FL1 values that a particle would need to fall within to be considered as a possible *Giardia* spp. cyst. Region R3 (on a FSC vs. SSC plot) defined an area within which particles with a size and internal complexity consistent with *Giardia* spp. would be located. Region R2 (on a FSC vs. FL1 plot) defined an area within which particles of the size and fluorescence of labelled *Giardia* spp. cysts would be located.

A portion of each sample was run at the HI flow rate setting for 2 minutes to collect a data file for each sample. The remainder of each sample was run at the LOW flow rate setting, and any particles falling within both regions R1 and R2 were sorted out. Sorted samples were passed through a transparent cell culture insert (12 well format, 1-μm pore size, Becton Dickinson, Franklin Lakes, NJ) to retain cysts and other particles. Following sorting, the membrane was cut out of the holder and placed on a glass slide. Seven-μL water was added to the membrane, and a cover glass placed on top. The cover glass was ringed with nail polish. The membrane was examined using an epifluorescence microscope, with viewing at 400x magnification, and any *Giardia* spp. cysts were identified and counted.

3.2 PARASITE PRODUCTION AND PURIFICATION

3.2.1 C. parvum

C. parvum used for recovery trials was commonly referred to as the Iowa strain. Oocysts were isolated from the faeces of infected neonatal Holstein calves according to Belosevic et al. (2000) via sieves followed by cesium chloride (CsCl) density gradients and centrifugation.

Prior to layering the density gradients, the following stock solutions were initially made. Tris-EDTA consisted of 500 mL deionised water with 3.94 g Tris-HCl, 1.46 g EDTA and a pH of 8.0. A CsCl stock solution (Cat#19-83125-5, Aldrich) was made by dissolving 180 g CsCl in 100 mL deionised water. The following working stocks were then prepared using the above solutions: 1.4, 1.1 and 1.05 g/mL CsCl. A discontinuous CsCl gradient was made in a 40 mL Beckman polyallomer centrifuge by gently layering 9 mL of the 1.1 g/mL CsCl stock onto 9 mL of the 1.4 g/mL CsCl working stock. The calf faeces, after having passed through several sieves, were then gently layered on top of the CsCl gradient and spun at 16,000 x g for 60 minutes. The highest dense band that contained the purified oocysts was removed and washed twice. For a more complete methodology see Belosevic et al. (2000).

Oocysts were used for up to 90 days post isolation.

3.2.2 G. lamblia

G. lamblia cysts (W.B. strain) were produced *in vitro* by an adaptation of the protocol outlined by Belosevic et al. (1983). Trophozoites were stored at –80°C in a 10 percent DMSO solution and were cultivated in the following Diamond's TYI-S-33 medium:

Trypticase-peptone	10 g
Yeast extract	5.0 g
Dextrose	5.0 g
Sodium chloride	1.0 g
L-cysteine hydrochloric acid	1.0 g
Ascorbic acid	0.1 g
K_2HPO_4	0.5 g
KH_2PO_4	0.3 g
Ferric ammonium citrate	0.01 g
Calf serum (3 months old-Hyclone lab)	50 ml
Vitamin additive (NCTC-13 from Gibco-BRL)	15 ml
Gentamycin (50 mg/L from Gibco-BRL)	1 ml
MilliQ water up to	500 ml

The pH was adjusted to 6.8 with sodium hydroxide and the media was filtered through 0.8 (Milli-Fil- P.F., Millipore Corp.) and 0.22 μm filters (Sterivex-GS filters with filling bell, Millipore Corp.).

When the culture had grown to confluence at 37°C, the trophozoites were encysted using Diamond's TYI-S-33 medium containing 10 mg/mL bovine bile and 0.5 mM lactic acid with a pH of 7.8. After 24 hours the culture was transferred to Diamond's TYI-S-33 medium by placing it on ice for 10 minutes and shaking the flask to loosen cysts. The contents were then spun at 400 x g for 10 minutes, decanted, and filled with growth media to transfer cysts into a cell culture flask where they were incubated for another 24 hours at 37°C. The cysts were once again spun at 400 x g for 10 minutes and the supernatant was decanted. Cysts were then suspended in MilliQ filtered water.

Cysts no older than four days were purified using CsCl density gradients. A CsCl stock solution with a specific gravity of 1.80 was made by adding about 360 x g of CsCl to a Tris-HCl EDTA Buffer and then adjusted it to a final specific gravity of 1.80 by adding additional CsCl or buffer until an average weight of 1 mL equalled 1.8 g. Distinct layers of 1 mL 1.144, 2 mL 1.072, and 1 mL 1.048 CsCl densities were layered and approximately 0.5 mL of *G. lamblia* stock was gently layered on top of the gradients and spun at 10,000 rpm (Beckman Ultracentrifuge) for 10 minutes.

The cysts used in the recovery trials were no older than 4 days.

Chapter 4

4 RECOVERY TRIALS

4.1 INTRODUCTION

Ideally detection assays should have high and consistent recoveries of (oo)cysts; that is, when seeding a source water with a known number of (oo)cysts, it is desirable to consistently detect as many of the (oo)cysts as possible during the final assay. However, protozoan detection methods have frequently yielded low recoveries of known number of (oo)cysts (Clancy et al. 1997b, Bukhari et al. 1998a, Young and Komisar 1999). In addition, detection assay recoveries are often not documented in studies, resulting in incomparable results and methods.

As stated previously, currently the most universal method is "Method 1623: *Cryptosporidium* spp. and *Giardia* spp. in Water by Filtration/ IMS/ FA" (USEPA 1999b). It is a performance-based method for detecting *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in natural waters. The method utilized in this study parallels Method 1623 with several modifications including the use of flow cytometry and FACS in place of straight microscopy (Chapter 3).

Four recovery trials were performed to assess the percent recoveries and variability of the detection methodology utilized in this study. The following chapter includes a background on protozoan detection methods and assays, the experimental design of the recovery trials, results and discussion.

4.2 BACKGROUND

Two protozoan detection methods, developed by the United States Environmental Protection Agency (USEPA), are ubiquitous within the water industry. The first method delineated by the USEPA, the Information Collection Rule (ICR) Method (USEPA 1995), consists of a spiral wound cartridge filter with a nominal porosity, Percoll-sucrose flotation to separate biological material from debris via buoyant density gradients, and an indirect immunofluorescent antibody (IFA) staining assay. Problems with the ICR Method include:

- low and variable percent recoveries of (oo)cysts;
- large false-positive and –negative rates;

- poor precision and accuracy;
- technical difficulty in performing the method (Clancy et al. 1999); and
- no specificity with regard to species differentiation; therefore the species of the organism cannot be determined (Watanabe 1996).

According to five studies that evaluated the performance of the ICR method, recoveries of both *Giardia* spp. and *Cryptosporidium* spp. (oo)cysts ranged from 0 to greater than 100 percent in spiked samples. Further, standard deviations were often equal to or greater than the mean with coefficients of variation that were multiples of the mean (Clancy et al. 1997a). Lastly, the detection limit for *Cryptosporidium* spp. in the ICR Method is much higher than the levels of oocysts that are expected to cause disease. For example, it is predicted that 10 to 30 oocysts per 100 L in treated water could lead to an outbreak. However, when up to 9000 oocysts were filtered in positive controls some laboratories could not detect a single oocyst (Clancy et al. 1997a).

Therefore "Method 1622: *Cryptosporidium* in Water by Filtration/ IMS/ FA" (USEPA 1999a) was developed. This method, which later gave rise to "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/ IMS/ FA" (USEPA 1999b) is a performance-based procedure; it allows product alternatives for the filtration, immunomagnetic separation (IMS), and staining thereby allowing constant improvement of recovery efficiencies.

However, the following problems with the former ICR method, according to Young (1999), also apply to Method 1623. Both methods have:

- temporal and spatial variability due to an unknown distribution of protozoa in the environment and treated water;
- indeterminate viability of detected (oo)cysts;
- uncertain specificity in the labelling procedure;
- sub-sampling error; and
- extrapolation from partial examination of the pellet by microscopy (Young and Komisar 1999).

Unfortunately, although Method 1623 gives rise to better recoveries than the ICR Method (Clancy et al. 1999), some problems still persist. Temporal and spatial variability of protozoa must be compensated by large numbers of samples; methods for assessing the viability of oocysts are still being gauged and created; sub-sampling error and partial sample analysis can only be offset by analyzing an entire sample which is only feasible by flow cytometry (which is not a standard procedure for Method 1623); and an antibody with a high degree specificity is still not universally used.

The recovery of parasites relies greatly on filtration and IMS products, protocols, and staining procedures. Bukhari and Clancy (1999) summarize much of the literature comparing the recovery efficiencies of different products. Below is a condensed review of filtration and IMS techniques, particularly as they relate to this study's products and techniques, followed by a literature review of current detection, viability and infectivity assays.

4.2.1 Filtration and Elution

The filtration and elution product utilized in this study was Filta-Max™ due to the following benefits. It is approved by the USEPA to sample a continuous flow of 50 L of raw water. Also, filter elution (washing) is performed by plunging the decompressed filter foam pads up and down in solution and by passing the suspension through membrane filters. This greatly reduces the amount of centrifugation steps to one or two prior to IMS. According to LeChevallier et al. (1995), depending on the centrifugation force, the loss of oocysts can be as high as 30 percent per centrifugation step. Filta-Max™ is also easily used in field sampling and is straightforward to transport.

Filta-Max™, in conjunction with Dynabeads® (Dynal, A.S., Norway), and IFA gave rise to oocyst and cyst mean recoveries of 39.7 ± 14.7 percent and 60 ± 5.5 percent, respectively from matrix spike samples (raw water samples) (Bukhari 2000). Filta-Max™ in conjunction with Puri-Max™ (Genera Technologies) gave rise to recoveries between 40 and 60 percent from drinking water volumes of 1,000 L containing 100 oocyst spikes (Parton et al. 1999).

4.2.2 Immunomagnetic Separation (IMS)

IMS is a relatively new technique that concentrates and purifies raw and drinking water concentrates. It gives rise to more reproducible and higher recovery results than Percoll-sucrose flotation, a technique utilized in the USEPA's older ICR Method (USEPA 1995). Several

IMS kits are now commercially available and are often compared within literature (Campbell and Smith 1997, Bukhari et al. 1998b, Rochelle et al. 1999, Zezulak et al. 1999, Bukhari and Clancy 2000). Generally the technique consists of labelling the protozoa with magnetic beads that adhere to a magnetic field. When they are removed from the magnetic field the protozoa and beads are released resulting in a cleaner, more concentrated solution.

Recoveries comparing sucrose flotation and IMS in raw water samples were 26.1 percent for IMS and 16.6 percent for flotation. Seeded backwash filter water recoveries were 9.1 and 5.8 percent for IMS and flotation, respectively (Di Giovanni et al. 1999). Further, IMS does not appear to affect oocyst infectivity (Rochelle et al. 1999, Couliette et al. 2000).

IMS can be improved by utilizing monoclonal antibodies with higher affinities for *C. parvum* and *G. lamblia* (Scandizzo et al. 1998, Ferrari et al. 1999). A flow cytometric analysis of anti-*Cryptosporidium* and anti-*Giardia* antibodies and their effect on method performance was presented by Hoffman et al. (1998).

The use of MACS IMS (Auburn, CA) system for concentrating *C. parvum* and *G. lamblia* from water samples is relatively new. (Oo)cysts are labelled with fluorescein isothiocyanate (FITC), which then combines with anti-FITC-labelled MicroBeads, and the protozoa are separated through a high gradient separation column. Qi Deng et al. (2000) recovered over 95 percent of fluorescent and infective *C. parvum*. Because of the size of MicroBeads in relation to oocysts (50 nm vs. oocysts of 4 to 6 μm in diameter (Qi Deng et al. 2000)) the beads do not need to be dissociated prior to analysis as they do not interfere with PCR, IFA excystation assays (Qi Deng et al. 2000) or with the fluorescence of FITC-labelled protozoa in flow cytometry assays.

4.2.3 Infectivity, Viability and Detection Assays

Assessing protozoan infections in human populations would be the most meaningful means of determining whether or not parasites are a danger to public health. As this is obviously not feasible, animal infectivity is the next best method of assessing if a human population is at risk. This assay provides the only irrefutable means of assessing whether or not (oo)cysts are infectious. However, animal infectivity assays are rarely used by laboratories due to their expense, labour and animal care facility requirements, and the variability of parasitic strains (Belosevic et al. 2000) thereby giving rise to several *in vitro* viability and infectivity assays. The following outlines current detection, viability and infectivity assays that are summarized later in Table 1.

4.2.3.1 *Fluorogenic Dyes*

The immunofluorescent antibody staining (IFA) assay is outlined by LeChevallier (1995), Campbell (1992), and both the ICR and 1623 methods (USEPA 1995, 1999b). IFA does not actually specify the viability or virulence of pathogens (LeChevallier and Norton 1995), rather, the dye permeability assay detects oocyst wall permeability which, in theory, is altered in a non-viable (oo)cyst (Jenkins et al. 1997). This technique relies on the inclusion or exclusion of 4',6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI). Some problems with this assay include:

- oocyst cell wall permeability increases to DAPI but not to PI with time;
- some oocysts appear to be permeable to both dyes (regardless of their viability); and
- cell wall permeability seems to be affected by temperature (Jenkins et al. 1997).

Again, the IFA technique only examines the permeability of the cell wall. A problem with the underlying assumption is that although the cell wall may be in tact, the organism may not be able to reproduce due to molecular or genetic changes in the cells that may have occurred due to stresses incurred by the environment or water treatment processes. Also, algae may interfere with microscopic examination of the samples due to the non-specific absorption of the dyes (Rogers et al. 1995).

The dye permeability assay tends to overestimate viability after UV and ozone treatment (Bukhari et al. 1999). Also, dye permeability assays and *in vitro* excystation give rise to correlated results (Campbell et al. 1992, Jenkins et al. 1997). No correlation between IFA and infectivity has yet been demonstrated (Neumann et al. 2000b).

4.2.3.2 *Excystation*

The *in vitro* excystation assay, as delineated by Campbell (1992), determines the ability of the oocyst to excyst to the trophozoite phase.

The assay mimics the conditions of the small intestine by exposing oocysts to reducing conditions such as bile salts and/ or the presence of a weak hypochlorite solution. It reflects the oocyst's potential for metabolism and attempts to extrapolate its potential for infectivity (Jenkins et al. 1997). However, although a cell may metabolize, it may not actually be able to reproduce.

The assay assumes that parasites that do not excyst are non-viable and non-infectious, however, according to Neumann et al. (2000a) non-excysted oocysts can infect neonatal CD-1 mice thereby suggesting that it is not a good indication of viability or infectivity. *In vitro* excystation also tends to overestimate the infectivity of oocysts after UV or ozone microbial reduction treatment (Bukhari et al. 1999).

Other problems with excystation are:

- large numbers of oocysts are required;
- it cannot discriminate between oocysts that have excysted spontaneously and those that are a result of exposure to stimuli;
- it is time intensive;
- there is a large degree of subjectivity amongst technician proficiency and conclusions (Korich et al. 1993); and
- as it relates to environmental samples, chemical, microbial or environmental stresses may affect results (Bukhari et al. 1999).

4.2.3.3 Flow Cytometry and FACS

In flow cytometry a suspension of particles is passed through a laser beam and the characteristics of light emitted as each particle passes through the laser are measured by the hardware and analyzed by associated software. Particles of increased size cause an increase in *forward-scattered* light (FSC). Particles of increased internal complexity cause an increase in *side-scattered* light (SSC). Some particles may emit fluorescent light (FL) when stimulated by the laser, either due to auto-fluorescence or because a fluorescent label (labelled during the IMS procedure) has been bound to them. The flow cytometer can detect fluorescent light of various wavelengths. If particles of interest cause a unique combination of FSC, SSC and FL emission, then they can be uniquely identified within a complex mixture of other particles at a rate of several thousand particles per second.

When a flow cytometer has a fluorescent activated cell sorting (FACS) mechanism, the flow cytometer is capable of sorting out particles of interest and shunting them to a collection apparatus where they are deposited on a membrane that can be examined microscopically for positive confirmation of particle identity.

The advantages of flow cytometry with FACS over microscopic examination are:

- entire sample analysis can be performed relatively quickly and with less labour than microscopy;

- it decreases subjectivity between observers thereby giving more representative results due to automated (oo)cyst detection;

- (oo)cyst may be confirmed relatively quickly by microscopy after sorting; and

- it improves the reproducibility and reliability of the microscopic counts (Compagnon et al. 1997).

Flow cytometry greatly increases the sensitivity in the detection of *Cryptosporidium* spp. and *Giardia* spp. relative to IFA microscopic analysis, from 35.3 to 94.1 percent and from 61.4 to 78.6 percent, respectively (Hoffman et al. 1997). Flow cytometry can also be used in conjunction with propidium iodide (PI) and therefore dead (oo)cysts in environmental samples can be detected (Medema et al. 1998).

Although flow cytometry requires a high capital cost and is time consuming, it is less labour intensive than microscopy for environmental samples and it provides entire sample analysis which gives rise to less error in analysis and lower detection limits; two critical points for microbial detection in natural and finished waters.

FITC-Staining

Labelling (oo)cysts with fluorescein isothiocyanate (FITC) is a detection assay; it determines the presence or absence of (oo)cysts. This staining assay depends on the specificity of the antibody. Incorrect detections may result due to the auto fluorescence of other particles such as algae; however, FACS in conjunction with verification via microscopy can help to alleviate this problem.

Environmental sample analysis with FITC may be problematic in certain situations as acidic pH significantly decreases the fluorescence emission of FITC molecules (Haulang 1996).

SYTO®-9 and SYTO®-59 Nucleic Acid Stains

According to Belosevic et al. (2000), SYTO®-9 and SYTO®-59 nucleic acid stains, in conjunction with automated flow cytometry, are "more reliable, highly reproducible, and significantly less time consuming than the standard epifluorescence microscopy assays". In addition there is also a relationship between *C. parvum* SYTO®-9 and SYTO®-59 assays and infectivity assay results (Belosevic et al. 2000, Neumann et al. 2000b).

Bukhari et al. (1998a) suggest that following UV or ozone microbial reduction treatment SYTO®-9 gives rise to a closer correlation with mouse infectivity than do SYTO®-59, DAPI, and excystation. However all four *in vitro* surrogates underestimate animal infectivity following UV and ozone treatment (Bukhari et al. 1998a). *C. parvum* stained with SYTO®-9 after treatment with ozone or monochloramine offers comparable results to animal infectivity (Belosevic et al. 2000).

As it relates to natural and treated waters detection, nucleic acid staining appears to be dependent on the type of disinfectant (especially free chlorine), pH, and temperature. *C. parvum* treated with chlorine dioxide or free chlorine and stained with SYTO®-9 do not give rise to correlated infectivity results (Belosevic et al. 2000) .

Although the SYTO-9 and –59 *in vitro* assays tend to underestimate the level of infectivity in mice, they may still be used as infectivity surrogates as long as they are compared to and correlated with infectivity trials for each application (Bukhari et al. 1999).

4.2.3.4 Cell Culture

Briefly, protozoa cell culturing involves excysting oocysts to trophozoites before infecting mammalian cell lines to determine their infectivity. Many cell lines are currently used for *in vitro* infectivity assays of *C. parvum* although no consensus has yet been reached regarding the most appropriate cell line (Rochelle et al. 2000). Work is still in progress to determine the most relevant cell line to use as it relates to infectivity.

Assessing infectivity by cell culture relinquishes the expense and ethical considerations of utilizing animals although the method is expensive, cell cultures are easily contaminated, and the method requires a large number of oocysts (Belosevic et al. 1997). In addition, the problems inherent with *in vitro* excystation are compounded with cell culturing disadvantages in this assay.

Slifko et al. (1998) suggest that the cell culture Foci Detection Method is a good alternative to animal infectivity for microbial reduction inactivation assays. The method was more sensitive than both IFA and excystation. However, it is unknown if this method has been used for raw and treated water protozoa infectivity.

Rochelle et al. (1997b) used reverse transcriptase-PCR to detect the heat shock protein 70 in *C. parvum* within infected cell cultures. Di Giovanni et al. (1998) detected the same protein by PCR in infected cell lines. Again, the problem with these methods, according to Neumann et al. (2000b), is that the problems associated with excystation are compounded on to those associated with *in vitro* cell culture assays. For example, oocysts that do not excyst are not detected by cell culture and excystation assays. Further, the preparation of the assay requires some activation and sterilization thereby potentially adversely affecting oocyst viability and infectivity (Neumann et al. 2000b). Finally, each cell line gives rise to a different degree of susceptibility to infection with *C. parvum* (Upton 1997).

It appears that if the cell culture assay were to be used as a standard environmental monitoring technique, the cell line and protocol would have to be standardized and results should be correlated with animal infectivity prior to standardization.

4.2.3.5 Polymerase Chain Reaction (PCR)

Several papers have touted using PCR in conjunction with a cell culture technique as a means of assessing the viability of (oo)cysts in natural and finished waters (Rochelle et al. 1997a, Rochelle et al. 1997b, Di Giovanni et al. 1999, Leon et al. 1999).

PCR has also been used as an assay unto itself. Chung et al. (1998, 1999) used PCR confirmed with nested PCR. Seeded samples gave rise to a high degree of correlation between IFA and PCR. Sensitivity, however, was low with a limit of 10^2 to 10^3 oocysts per 100 L of treated water (Chung et al. 1998). Rochelle et al. (1997b) tested eight pairs of PCR primers for the detection of *C. parvum* and *G. lamblia*. Detection sensitivities were from 1 to 10 (oo)cysts in purified preparations and from 5 to 50 (oo)cysts in spiked environmental water samples. Hallier-Soulier and Guillot (1999) were able to detect the presence of a single oocyst in a concentrate from 5 to 10 L of drinking water.

Kozwich et al. (1999) were able to detect 10 oocysts/L in treated water and 1 oocyst/L in raw water while using a reverse transcription-PCR assay and lateral flow detection for *C. parvum*. Their assay, from re-suspension of the pellet to detection was approximately 3 hours, compared to 5 hours generally required for IMS followed by IFA and microscopy

Rochelle et al. (2001) obtained sensitivities of one oocyst with nested PCR and conclude that Method 1622 combined with PCR-based genotyping can detect less than 10 oocysts per 10 L. They suggest that PCR should be used in conjunction with IFA and other detection techniques and should be used more as a method of identifying the species in question and consequently the source of contamination.

A small subunit (SSU) rRNA-based PCR-restriction enzyme length polymorphism (PCR-RFLP) technique for detecting and differentiating species of *Cryptosporidium* spp. was successful for Xiao et al. (2000). The study detected and differentiated *Cryptosporidium* spp. in storm water, wastewater, and surface water samples and determined that the method had a high sensitivity and specificity for *C. parvum* detection and differentiation.

According to Rochelle et al. (1997b):

> "The advantages of PCR include greater sensitivity, rapid analysis of many samples, relatively low cost, simultaneous detection of multiple pathogens, and the ability to discriminate between species and strains if suitable primers are detected."

Problems with PCR include:

- low reliability of amplification with low input molecule numbers;
- interfering substances commonly found in natural waters;

- the method consistency is affected by the reduction of DNA on spin columns, the efficiency of oocyst lysis steps, and centrifugation (Chung et al. 1999); and

- it is highly dependent on the primers that are utilized (Leon et al. 1999).

4.2.3.6 Fluorescent In-situ Hybridization (FISH)

The fluorescent *in-situ* hybridization (FISH) technique consists of targeting a specific sequence on the 18S ribosomal RNA (rRNA) of *C. parvum* with a fluorescent-labelled oligonucleotide probe (Vesey et al. 1997). The assay relies on the premise that rRNA has a short life span in non-viable oocysts.

This viability assay, in conjunction with IFA staining, thereby provides species specificity and rapid processing time (Deere et al. 1998). However FISH does not provide any information on infectivity and FISH assay results correlate well with excystation, which does not appear to be a reliable technique. Also, FISH must be combined with immunofluorescent staining because FISH-stained oocysts do not fluorescence brightly enough in environmental water concentrates (containing autofluorescent algae and mineral particles) (Vesey et al. 1998).

4.2.3.7 Infectivity

The use of animals, such as gerbils, to simulate *G. lamblia* infections in humans is discussed in Faubert and Belosevic (1990) and the method is outlined in Belosevic et al (Belosevic et al. 1983). Finch et al. (1993) developed a protocol using mice to simulate *C. parvum* infections in humans. *In vivo* (animal infectivity) trials are the only indisputable method that can determine the infectivity of parasites. However, as previously mentioned drawbacks to infectivity include expense, labour, ethical considerations, animal care facility requirements, and the variability of parasitic strains and variable animal susceptibility (Belosevic et al. 2000). The use of animal infectivity in environmental monitoring also presents another problem.

The median infectious dose (ID_{50}) of *C. parvum* for CD-1 neonatal mice ranges between 69 and 145 oocysts. This is based on three years of neonatal mouse infectivity data with oocysts obtained from 10 calves (Korich et al. 2000). The ID_{50} for gerbils infected with *G. lamblia* ranges between 245 and 250 (Schaefer III et al. 1991). Due to the low numbers of (oo)cysts that are typically found in natural and treated waters, infectivity should not be used quantitatively but rather qualitatively where large numbers of (oo)cysts exist.

Table 1: Comparison of *C. parvum* and *G. lamblia* viability, infectivity and detection assays as they relate to waterborne environmental samples. Table was adapted from Belosevic et al. (1997, 2000).

Assay	Advantages	Disadvantages
Animal infectivity	Only irrefutable method of determining the infectivity of (oo)cysts Can be correlated with human infectivity	Expensive Labour intensive Requires animal holding facilities Not all *C. parvum* and *G. lamblia* genotypes infect mice or gerbils, respectively High ID_{50}'s therefore requiring large numbers of (oo)cysts
Cell culture viability/ infectivity assays	Can measure viability and infectivity without animals	Inaccurate and expensive Low infectivity and therefore requires large numbers of oocysts Cultures are easily contaminated creating difficult infection assessment
Fluorogenic dyes (IFA)	Simple to use Inexpensive	Does not measure viability nor virulence Subjectivity between microscopic examiners Oocyst wall permeability affected by temperature and time Algae may interfere with microscopic examination Some oocysts appear to be permeable to both DAPI and PI, regardless of viability
***In vitro* excystation**	Simple to use Inexpensive	Labour intensive and inaccurate Dead parasites can excyst Does not measure infectivity
Nucleic acid dyes	Simple to use	Labour intensive

Assay	Advantages	Disadvantages
by microscopy	Inexpensive Reliable depending on microbial reduction method	Does not measure infectivity High detection limit Partial sample analysis increases error in environmental samples
Nucleic acid dyes assessed by flow cytometry	Simple to use Reliable Rapid assessment of viability Nucleic acid staining can be related to animal infectivity Entire sample analysis (Oo)cysts may be confirmed by microscopy SYTO-9 and SYTO-59 results may be correlated with infectivity results in some situations	Does not measure infectivity Expensive equipment required Treated water correlation with animal infectivity relies on method of microbial reduction
Molecular techniques such as PCR etc.	Sensitive Enables speciation	Does not measure infectivity Assesses only a small sample of the parasite population Requires molecular lab set-up and expertise Inadequate probes influence specificity Method is affected by several inhibitors

4.2.4 Indicators

Direct protozoa detection is labour intensive and often provides low or variable recoveries due to limited testing procedures as reviewed in the previous sections. To date, no test can quickly and effectively detect *Cryptosporidium* spp. and *Giardia* spp. in treated and natural waters. Therefore there have been several attempts to correlate other water quality parameters with the presence of protozoa.

Although correlations between either turbidity and/ or indicator organisms, such as total and faecal coliforms, have been found (LeChevallier et al. 1991a, LeChevallier et al. 1991b, LeChevallier and Norton 1992, Chauret et al. 1995), other studies have found no correlation between turbidity and/ or indicator organisms (Braidech and Karlin 1985, LeChevallier and Norton 1992, Chauret et al. 1995).

In a study by Rose et al. (1991) there was no correlation between either *Giardia* spp. and *Cryptosporidium* spp. and bacterial indicators. However, the presence of oocysts and cysts were significantly correlated. Payment (1999) suggests that due to the higher sensitivities of indicator organisms (such as *E. coli* (Fricker and Clancy 1998)) to chlorine, they may not be effective indicators of the presence of other pathogenic microbes.

Cryptosporidium spp. concentrations cannot be predicted using turbidity and particle counts in raw or treated waters. However, according to Edzwald and Kelley (1998), both parameters are important for recognizing when particles pass through treatment processes. Filtered water turbidity of less than 0.1 NTU and particle counts of 50/mL or less are good indicators for controlling *Cryptosporidium* spp. (Edzwald and Kelley 1998). Nieminski and Ongerth (1995) found that with conventional treatment and direct filtration, particle counts of (oo)cyst-sized particles is a reliable indicator of (oo)cyst removal. Turbidity and heterotrophic bacteria removal did not correlate well with (oo)cyst removal although the log removal of turbidity may be used as a rough estimate of (oo)cyst removal.

4.2.5 Detection Overview

A problem inherent with all protozoan environmental detection techniques is the ability to spike solutions with exact numbers of organisms, especially because most detection work is performed with very small numbers – in the order of 500 or less. Reynolds et al. (1999) developed a spiking technique by counting a "seed dose" with a flow cytometer with cell sorting capacity (Becton Dickinson FACScalibur flow cytometer). They created doses of 100 oocysts with a mean of 99.5 oocysts with a standard deviation and percent coefficient of variation of 1.1; thus the technique creates far more accurate doses than conventional microscopic techniques, eliminating a substantial source of error which is inevitably compounded throughout the assay.

Using this seeding procedure; calcium carbonate flocculation/ centrifugation of 10 L raw water samples; Dynal IMS (Dynabeads anti-*Cryptosporidium*, product no. 730.0 Dynal AS, Oslo, Norway); staining with FITC-mAb/ DAPI; and analyzed by "ChemScan Detection of *Cryptosporidium* oocysts on a Membrane filter Kit," (Chemunex, Maison Alfort, France), Reynolds et al. (1999) obtained recoveries of oocysts ranging between 82.3 and 86.3 percent.

This study by Reynolds et al. exemplifies that a high degree of sensitivity in (oo)cyst detection are possible with improved technologies and methodologies. Species differentiation can be assessed by PCR technologies. Infectivity can be assessed to some extent by cell culture methods and ultimately by animal infectivity, and it is anticipated that viability may eventually be examined through FISH techniques. Although widely used, DAPI/PI staining and *in vitro* excystation do not appear to be reliable techniques. Again, there is a large amount of variability in recovering (oo)cysts which is, in part, associated with each water matrix and the volume of water filtered.

4.3 EXPERIMENTAL DESIGN

The following recovery trial filtration procedure was adapted from Bukhari (2000). A known number of oocysts and cysts were pipetted into a polycarbonate carboy filled with 10 L of water. The pipette tip was rinsed with PBST prior to cyst inoculation. The carboy was stirred via magnetic stir bar for one minute prior to and throughout filtration. The carboy was then drained to approximately 1 L through Tygon R-3603 tubing, a Simer portable utility pump, Filta-Max filter and housing and finally through an ABB water meter at a flow rate of 3 L/min. The carboy was filled to 10 L and drained again to 1 L several times until approximately 50 L of raw water was passed through while allowing the pump to work continuously. Finally the carboy was rinsed with approximately 2 L deionised water, which was also filtered at 3 L/min.

Filters were contained within filter housings and stored at 4°C for up to 24 hours prior to elution. See Chapter 3 for the remainder of the procedure.

4.4 RESULTS

Table 2 summarizes the pH and turbidity of the raw waters utilized in the recovery trials. Both water samples A and B were taken from Saddle Lake water treatment plant water intake which consisted of a mixture of Saddle Lake raw water and recycled backwash water. Sample water A was taken on August 30, 2001 and used in recovery trials 1 and 2, and sample water B was taken on September 20, 2001 and used in trials 3 and 4. Trials 1 and 2 were performed on a different day than trials 3 and 4 to assess variability between days. The water samples appear to be reasonably consistent in pH and turbidity.

• Table 2: Turbidity (NTU) and pH of the Saddle Lake water treatment plant intake used in the recovery trials.

	A	B
pH	9.7	9.3
Turbidity	1.7	5.0

Tables 3 and 4 display actual (oo)cyst counts obtained from initial seedings. These initial seedings were created simultaneously with carboy spikes during the recovery trials. They were later stained with FITC and then sorted by flow cytometer and finally counted by microscopy. The average from 1A and 1B divided by four was used in determining the percent recovery for trial 1. Likewise the average from 2A and 2B divided by four was used in determining the percent recovery for trial 2, etc. The seeding counts were divided into four because the recovery trial concentrates were divided into four prior to staining in accordance to the protocol implemented in the Saddle Lake samples.

• Table 3: *G. lamblia* initial seeding counts and final percent recoveries.

Initial Seedings		**Percent Recoveries**		
Sample	**Confirmed Cysts**	**Sample**	**Confirmed Cysts**	**Percent Recovery**
1A	1092	*Negative Control*	*0*	
1B	1336			
Mean/4	303.5	1	166	55%
2A	1594			
2B	1398			
Mean/4	374	2	77	21%
3A	1809	*Negative Control*	*0*	
3B	1189			
Mean/4	374.75	3	209	56%
4A	1559			
4B	1093			
Mean/4	331.5	4	160	48%

• Table 4: *C. parvum* initial seeding counts and final percent recoveries.

Initial Seedings		**Percent Recoveries**		
Sample	**Confirmed Oocysts**	**Sample**	**Confirmed Oocysts**	**Percent Recovery**
1A	399	*Negative Control*	*0*	
1B	449			
Mean/4	106	1	25	24%
2A	469			
2B	599			
Mean/4	134	2	7	5%
3A	384	*Negative Control*	*0*	
3B	303			
Mean/4	86	3	15	17%
4A	243			
4B	216			
Mean/4	57	4	19	33%

Tables 3 and 4 also summarize the waterborne protozoa recovery trials. The average percent recoveries for *G. lamblia* and *C. parvum* were 44.8 and 19.9 percent, respectively. There was a large degree of variability between trials as recoveries ranged between 21 and 56 percent for *G. lamblia* and between 5 and 33 percent for *C. parvum.* The spread of data is fairly consistent between the trials of both species, which is about 30 percent. The variable recoveries were also reflected by large coefficients of variation: 37 and 59 percent for *G. lamblia* and *C. parvum*, respectively (Table 5).

• Table 5: Summary of *G. lamblia* and *C. parvum* recovery trials.

	G. lamblia	***C. parvum***
Mean of Trials 1,2,3,4	45%	20%
Standard Deviation	16%	12%
Coefficient of Variation	37%	59%

- Table 6: Comparison between initial seedings and recovery trials of the ratio of microscope count (actual count) over flow cytometer count expressed as a percentage (*ND=not done).

Initial Seedings		**Recovery trials**	
Giardia lamblia	**Percentage of Actual vs. FACS Counts**		**Percentage of Actual vs. FACS Counts**
1A	70 %		ND*
1B	84 %		ND
2A	90 %		ND
2B	86 %		ND
3A	84 %	1	49 %
3B	61 %	2	26 %
4A	81 %	3	41 %
4B	76 %	4	35 %
Mean	**79.00 %**		**37.75 %**
Standard Deviation	**9.5 %**		**9.7 %**
Cryptosporidium parvum			
1A	47 %		ND
1B	65 %		ND
2A	62 %		ND
2B	72 %		ND
3A	64 %	1	27 %
3B	58 %	2	33 %
4A	57 %	3	33 %
4B	56 %	4	49 %
Mean	**60.13 %**		**35.50 %**
Standard Deviation	**7.4 %**		**9.4 %**

4.5 DISCUSSION

It is highly probable that a large amount of variability is inherent in percent recoveries due to diverse water matrices caused by variations in source waters, seasons, and, in treated waters, by applied microbial reduction methods. For an accurate estimate of an actual recovery as it relates to a detection sample it would be desirable to assess the mean recovery and its variability through spiking samples of identical source water in the same quantity as the detection sample and process them at the same time. However, as this was not feasible due to labour and expense, and because this would greatly increase the potential for contamination, the above recovery trials were performed as an independent assessment of actual recoveries.

Therefore, due to the inherent variability between water matrices, these recovery trials may be used as an indication of actual recoveries within the drainage basin. Using the current techniques, recoveries within the drainage basin are likely low and variable. The raw water used in the recovery trials was of average to high turbidity, and of a basic pH in comparison with Saddle Lake samples. Packed pellet volumes, which are good indicators of the amount of debris in the concentrate prior to IMS, tended to be less than or equal to 0.1 mL for all recovery trials, which was comparable with the vast majority of Saddle Lake samples. Nevertheless, these results should primarily indicate that the current protozoa detection assay should be further optimized to generate higher recoveries of both oocysts and cysts with smaller coefficients of variation. This conclusion was drawn, in part, from the USEPA's Method 1623 (USEPA 2001) acceptance criteria and from several studies that obtained higher, less variable recovery results (Reynolds et al. 1999, Bukhari 2000).

In comparing the ratio of observed (oo)cysts and the flow cytometer counts (expressed as percentages), it is clear that the FACS is picking up many more indeterminate particles from the much dirtier, raw water samples. This was reflected by averages of 79 and 60 percent from the initial seedings compared with 38 and 36 percent from the recovery trial for *G. lamblia* and *C. parvum*, respectively. The percent of actual (oo)cysts viewed on the membrane vs. the count according to the FACS was much larger for the cleaner seeded samples, compared to the dirty raw water recovery samples.

The gated regions used in the recovery trials was slightly smaller than gate used in the Saddle Lake samples. This would not likely affect results as the gates had been refined for ease of manual microscopy through working with Saddle Lake samples.

Variation in recovery results in raw water may be due to their matrices and chemistry which vary seasonally in part due to summer algal growth, seasonal runoff and mixing, anaerobic activity under ice cover, and human and animal influence within the water. Variable or low recoveries may also be due to any of the number of steps used in this project's methodology. Table 7 summarizes potential problems within this study's techniques and means of possibly diagnosing or improving the techniques.

The seeding technique must be improved to reduce initial variability (Tables 3 and 4) that is compounded throughout the methodology. A large number of (oo)cysts are likely lost during filtration and elution procedures decreasing recoveries and likely increasing variability. Although other studies have shown higher recoveries with Filta-Max, the protocol may be improved. The staining procedure may need to be optimized according to eluate pellet size or initial source water qualities such as turbidity or particle counts. Further, although this would likely increase FACS operational time, IMS may be omitted from the process. Presently the concentrates are being concentrated and cleaned twice - by IMS and by FACS. This redundancy may not be necessary. Omitting IMS from the detection method can assess the need for IMS when using FACS to sort samples. Also, because MACS products have not been used before in waterborne protozoa detection, to the author's knowledge, they may not be conducive to high recovery results. Should IMS be omitted, the concentrate must still be filtered through a 10 μm pre-separation filter so that the concentrate will not block flow cytometer tubing.

• Table 7: Summary of possible sources of variability and low recoveries of waterborne protozoa as they relate to the current study's detection method.

PROCEDURE	PROBLEM	SOLUTION
SEEDING • *A large source of variability* as per *Table 4 that is compounded throughout the analysis*	How should the initial seeding be determined? There is variability between DIC[10] microscope counts, fluorescent microscope counts, and FACS counts	Perform DIC, fluorescence, and FACS counts on the same stock solution & compare results
	Need a more accurate and precise way of producing the seedings	Create seedings by FACS (Reynolds et al. 1999)
FILTRATION • *Filta-Max has given rise to higher and less variable recoveries (Bukhari 2000)*	Filtration and Elution with Filta-Max has produced higher & less variable recoveries (Parton et al. 1999, Bukhari 2000)	Not likely the primary source of variability and very low recovery although the washing step might be enhanced by using a larger volume of PBST
SUB-SAMPLING • *Each sample is divided into 4 sub-samples after filtration for 4 different analyses*	Sub-sampling can greatly increase error (Young and Komisar 1999)	Compare subsample vs. entire sample recoveries Do not subsample

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[10] DIC denotes "differential interference contrast."

PROCEDURE	PROBLEM	SOLUTION
STAINING • *Staining has not been optimized with dirty and clean concentrates*	Are antibody concentrations adequate in concentrates? • E.g: Are antibodies attaching to other particulates in the concentrates?	Filter both dirty and clean samples and then seed concentrate with protozoa. Assess by flow cytometer. Correspond antibody concentrations with eluate pellet size
	Are oocysts being stained optimally?	Incubate *Cryptosporidium* spp. after exposure to antibody
	Can staining be optimized?	Continuously shake tubes during incubation
	Anti-*Giardia* and anti-*Cryptosporidium* antibodies have variable specificities and bonding abilities (Hoffman et al. 1998, Weir et al. 1998).	Perform specificity analyses with various species of *Giardia* and *Cryptosporidium* to determine which antibody has the highest specificity.
IMS • *Likely a large source of variability and low recovery as MACS is not commonly used in Method 1623.*	Is there a loss of protozoa due to high pressures within the MACS column? Is MicroBead adhesion sufficient? Is IMS necessary?	Try omitting IMS from procedure.
	Are MicroBeads binding non-specifically to other particulates?	Filter both dirty and clean samples and later seed concentrate with protozoa. Perform IMS and assess by FACS.
FACS • *Although there is potential for recovery loss due to clumped*	Are initial data files necessary?	Sort entire sample.[12]
	Do high and low flow rates give rise to equal recoveries?	Compare recoveries at high and low flow rates.

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[11] The percent of observed (oo)cysts in the initial seedings in table 6 indicates that FACS is sorting a high number of actual (oo)cysts. It is therefore probable that low recoveries of (oo)cysts are not likely being lost in large amounts due to FACS.

[12] This applies to Saddle Lake drainage basin samples only. Recovery trials were entirely sorted.

PROCEDURE	PROBLEM	SOLUTION
due to clumped (oo)cysts, FACS is likely sorting properly[11].	Are the current gates giving rise to the highest percent recoveries?	Compare several different gates, sort by FACS and assess by microscopy.

(Oo)cyst recoveries may be affected by (oo)cyst survival within different water matrices due to the presence of ammonia (Jenkins et al. 1998), salinity (Simmons and Sobsey 2000), pH, temperature (Naik et al. 1982), and high initial concentrations of heterotrophic bacteria (Heisz et al. 1997). Although Heisz et al. (1997) found that water chemistry was not very important for the viability of protozoa. It is possible that (oo)cysts may not survive well under conditions of increased concentrations of particulate matter and other microbes, in which case water samples may need to be processed more rapidly rather than over a period of four to five days.

Filta-max™ has been used in several studies and has shown higher and more consistent recoveries than those obtained in this study. Method 1623 performed with 50 L filtration of water by Filta-Max™, IMS with Dynal, and microscopic examination of fluorescent antibody staining gave rise to initial precision recovery[13] (IPR) of oocysts from 41.9 to 62.4 percent with mean recoveries of 50.6±5.6 percent (Bukhari 2000). The mean *Giardia* spp. cyst recovery was 57.6±9.6 percent with a range from 40.9 to 70 percent. Matrix spike[14] (MS) recoveries of *Cryptosporidium* spp. ranged from 19.5 to 54.5 percent with a mean recovery of 39.7±14.7 percent. *Giardia* spp. cyst recoveries from matrix spikes ranged from 54.5 to 69.1 percent with a mean of 60±5.5 percent. Parton et al. (1999) also obtained high although variable recoveries of oocysts within waters of variable turbidities by using Filta-max, Puri-max for IMS, and standard IFA microscopy.

Miltenyi Biotec's version of IMS, using MACS separation columns and MicroBeads, has not previously been used for waterborne protozoa detection to the author's knowledge. One study examined immunomagnetic separation of *C. parvum* oocysts using MACS MicroBeads™ and high gradient separation columns with apple juice, homogenized milk and deer manure. The IMS-PCR technique could detect as few as 10 oocysts from 100 mL apple juice or milk and IMS-IFA could detect 100 oocysts from 1 g deer manure (Qi Deng et al. 2000). The results obtained indicate that the FITC fluorescence and oocyst infectivity were retained after binding FITC with anti-FITC MicroBeads. Due to a general lack of information with regard to this product's performance in protozoa detection, the utilization of MACS products should either be omitted or optimized for further sample analysis.

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[13] Initial precision recovery refers to the percent recovery of (oo)cysts after spiking a volume of reagent water with (oo)cysts in accordance with Method 1623 (USEPA 1999b).

[14] Matrix spike recoveries are determined by spiking and analyzing a sample of field water to determine the effect of the matrix on the method's oocyst and cyst recovery (USEPA 1999b).

FACS has been used in protozoa water detection primarily for three reasons:

- to separate the protozoa from the majority of other debris within the concentrated water sample;
- to facilitate microscopic observation both in terms of the labour and reliability of the assessment; and
- to allow a greater volume of sample to be analyzed (Compagnon et al. 1999).

Research carried out in two laboratories in France and the Netherlands by Compagnon et al. (1999), indicates that FACS gives rise to more efficient, reliable, and higher or equivalent recovery rates when compared with an IMS technique. Both oocyst and cyst counts were more repeatable with FACS than with direct microscopic counts. Flow cytometry greatly increases the sensitivity in the detection of (oo)cysts relative to IFA microscopic analysis (Hoffman et al. 1997).

Chapter 5

5 SADDLE LAKE DRAINAGE BASIN WATER QUALITY

5.1 INTRODUCTION

Treated and raw water was sampled for protozoa due to concerns of Saddle Lake First Nation residents. Treated water was assessed for protozoa occurrence and infectivity to determine if the population was at risk of contracting cryptosporidiosis and giardiasis. Various raw water samples from throughout the Saddle Lake drainage basin were also tested for protozoa in an attempt to determine potential sources of the pathogens. Total and faecal coliforms, heterotrophic plate counts (HPC), turbidity and pH were also assessed to examine correlations between the water quality parameters.

The following chapter includes background literature on sources and occurrence of protozoa, outbreaks of cryptosporidiosis and giardiasis, public health risks, water quality legislation and management, and Saddle Lake water quality management. Experimental design, results and discussion will follow the background information.

5.2 BACKGROUND

5.2.1 Sources of (Oo)cysts

Sources of both *C. parvum* and *G. lamblia* (oo)cysts include the following[15]:

- human beings:
    - sewage discharge, septic tanks, latrines, etc.;
    - faecal run-off from soil into streams, rivers, etc.;
- infected animals
    - livestock pastures (especially from calves and lambs (Crockett and Haas 1997));
    - wild animals; and

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[15] Adapted from Smith *et al.* (1995).

- domestic animals.

Human activities that can increase the risk of waterborne giardiasis and cryptosporidiosis include:

- disposal of faeces and effluents from farms or feedlots;
- poorly constructed slurry stores;
- slurry spraying and manure spreading on pasture lands;
- intensive livestock rearing such as feedlots; and
- poor disposal of contaminated backwash sludge from water treatment plants (Smith et al. 1995, States et al. 1997).

(Oo)cyst source and occurrence have been greatly contested in a variety of studies. However, it is highly likely that cattle, sewage, industrial effluents, and wildlife contribute to the occurrence of *C. parvum* and *G. lamblia* in natural waters (Mann et al. 1986, Pohjola et al. 1986, LeChevallier et al. 1991a, Sischo et al. 2000).

5.2.2 **Occurrence**

Note that the following examples of occurrence are not directly comparable or equivalent due to the variability of detection methods and their recoveries. This list is not exhaustive and simply represents examples of (oo)cyst occurrence.

5.2.2.1 C. parvum

Cryptosporidium spp. appears to be ubiquitous throughout the world. It was detected in 74 of 85 (87 percent) of raw water locations primarily in the eastern United States, California, and Alberta (LeChevallier et al. 1991b). The geometric mean of the protozoa was 2.70 oocysts/L. LeChevallier et al. (1991) suggests that there is a clear linear relationship between *Cryptosporidium* spp. densities and levels of source water protection, with concentrations increasing from protected watersheds, to those with limited access, to recreational use, to agricultural use, sewage discharge, and industrial-urban discharge (LeChevallier et al. 1991b).

In Canadian drinking water supplies *Cryptosporidium* spp. oocysts were present in 6.1 percent of raw sewage samples, 4.5 percent of raw water samples, and 3.5 percent of treated water samples as determined by immunofluorescence techniques (Wallis et al. 1996). Oocysts were also routinely detected in the Ottawa region in concentrations ranging from less than 1 to 225 oocysts/ 100 L (Chauret et al. 1995). Fifty five percent of 257 water samples from the United States were assessed for *Cryptosporidium* spp. oocysts with an average concentration of 43 oocysts per 100 L (Rose et al. 1991).

Oocysts have been found in German raw and finished waters (Karanis et al. 1998), Taiwanese waters (Hsu et al. 1999), and US Virgin Island cisterns (Crabtree et al. 1996), among other countries.

For a more comprehensive review of the oocyst occurrence, see Lisle and Rose (1995).

5.2.2.2 G. lamblia

Giardia lamblia has been detected worldwide in both treated and raw waters. Studies have shown that cysts were present in 16.9, 19 and 22 percent of treated drinking water samples in the United States, Scotland and Spain, respectively (Smith et al. 1995). Rose et al. (1991) found 16 percent of 257 water samples were positive for *Giardia* spp. with an average concentration of 3 cysts per 100 L in the United States.

Giardia spp. were found in 69 of 85 (81.2 percent) of raw water samples with a detectible geometric mean of 2.77 cysts/L primarily from the eastern United States but also in California and Alberta (LeChevallier et al. 1991b). Cysts were routinely detected in the Ottawa region in concentrations of less than 1 to 52 cysts/ 100 L (Chauret et al. 1995).

Both cysts and oocysts appear frequently in travelers (Jokipii et al. 1985) and in day cares (Pickering and Englekirk 1990).

5.2.3 Outbreaks

An incident of protozoan waterborne outbreaks is more probable than an episode of bacterial or viral pathogen outbreak due to (oo)cyst resistance to common disinfection techniques (Lisle and Rose 1995). For example, in 1991 and 1992, 17 states and territories voluntarily reported 34 outbreaks of waterborne drinking water diseases. Either *G. lamblia* or *C. parvum* were responsible for seven of the eleven outbreaks for which the etiological agent was determined. The remaining four were a result of hepatitis A, *Shigella sonnei*, or chemicals (Moore et al. 1994).

Studies have shown a general reluctance to seek medical assistance with symptoms of diarrhoea. Because people only tend to consult with a physician when symptoms are severe or drawn out, it has been surmised that incidences of cryptosporidiosis are greatly underestimated. There is also a lack of stool sampling in the event that a patient does report symptoms of diarrhoea (Berkelman 1994, MacKenzie et al. 1994).

5.2.3.1 C. parvum

According to Lisle and Rose (1995), as of 1995 there had been five reported outbreaks of cryptosporidiosis in the USA and eight in the United Kingdom. The first was a groundwater outbreak that occurred due to a sewage-contaminated well in Bruan Station, Texas in 1984. Of 1791 households, approximately 34 percent were infected despite chlorination treatment techniques (D'Antonio et al. 1985). Later, in 1987, in Carrollton, Georgia, 39 percent of 13,000 people had gastroenteritis due to *Cryptosporidium* spp. The source of infection was a treated surface water source that had experienced treatment deficiencies, as was the cause of the proceeding outbreaks (Hayes et al. 1989).

In Pennsylvania 551 individuals were infected by a groundwater source in 1991 (Moore et al. 1993). In 1992, Jackson County, Oregon, 15,000 infections arose from the exposure of 160,000 to a spring and surface water source (Leland et al. 1993). Perhaps the most significant cryptosporidiosis outbreak to date occurred in 1993 in Milwaukee, Wisconsin. Of an exposed population of 1,600,000, an estimated 403,000 were infected due to a conventional water treatment system failure (MacKenzie et al. 1994). The Milwaukee outbreak created the greatest awareness of waterborne outbreaks of cryptosporidiosis. The outbreak appeared to be linked to an increased turbidity in the finished water.

Clark County, Nevada experienced an outbreak of cryptosporidiosis in 1994. There were 78 confirmed cases of cryptosporidiosis, of which 61 were HIV-positive adults. Twenty of the 32 individuals that had died by June of HIV-related causes had cryptosporidiosis on their death certificates (Goldstein et al. 1996).

From 1983 to 1991 there were eight reported outbreaks in the UK that infected a total of 887 individuals. The source waters were primarily surface and spring water (Lisle and Rose 1995). In Bradford, England, 125 people contracted cryptosporidiosis from a single water treatment plant supply (Atherton 1995).

5.2.3.2 G. lamblia

A collaborative disease outbreak surveillance system performed by the Centers for Disease Control and Prevention (CDC), the USEPA, and the Council of State and Territorial Epidemiologists was compiled for the period of 1976 to 1994. The results indicated that *Giardia lamblia* was the causative agent for 21.8 percent of 414 waterborne disease outbreaks, resulting in 15,839 illnesses (or 3 percent of 528,757 illnesses) (Schneider 1998).

In November 1981, a giardiasis outbreak occurred in Colorado. It resulted from a lack of chemical pre-treatment, faulty backwashing procedures, and poor application of natural water to the filters (Braidech and Karlin 1985).

Waterborne cysts in Edmonton, Alberta may have caused an outbreak of gastrointestinal disease in the mid 1980's. Water was not tested, therefore the source was inconclusive, however, over 800 people were infected by G. *lamblia* (Wallis et al. 1986).

5.2.4 Public Health Risks

Determining the risk of protozoan infection from natural and treated waters is a difficult and controversial task. The risk of obtaining a waterborne infection is essentially derived from assessing five variables:

- concentration of (oo)cysts in natural source waters;
- percent recovery of the protozoan detection method;
- viability of recovered (oo)cysts;
- removal of organisms in the water treatment processes; and
- daily consumption of the non-boiled potable water (Teunis et al. 1997).

Because of the variability in each of these variables, there is a great variability in waterborne protozoa health risk assessments. In addition, risk assessment variability and unreliability is increased due to unreported cases of cryptosporidiosis and giardiasis, which are likely due to a general unwillingness to report diarrhoeal symptoms and from a failure to sample stool when a patient with gastroenteritis visits his/her doctor (Berkelman 1994, MacKenzie et al. 1994). This likely results in an underestimation of cryptosporidiosis and giardiasis, thus increasing error in assessing outbreaks and in back calculating health risks from protozoan outbreaks.

5.2.4.1 C. parvum

According to a study by DuPont et al. (1995), a low dose of C. *parvum* is able to cause infection in a healthy adult with no serological evidence of prior infection. A dose of 30 oocysts gave rise to an infection in twenty percent of the healthy individuals. Eighty-eight percent of healthy individuals were infected from a dose of 300 or more oocysts (14 out of 16 volunteers). The calculated ID_{50} for the given Iowa strain of C. *parvum* was 132 oocysts (DuPont et al. 1995).

The estimated oocyst level sufficient to have caused the outbreak in Milwaukee is 0.42 to 4.5/L (MacKenzie et al. 1994). This result is derived from compiling detection results from finished distribution system waters and from samples of ice that were frozen during the outbreak. A correction factor was then applied to compensate for the number of oocysts lost in the sampling procedure. This gave rise to a geometric mean oocyst concentration of 0.79 oocysts/L in the drinking water (Haas and Rose 1994).

Haas and Rose (1995) estimated an action level for water utilities by comparing the concentrations of C. *parvum* oocysts in treated water during recognized outbreaks with waters where no outbreaks have occurred. The study suggests that when a drinking water sample contains 10 to 30 oocysts/ 100L or more, the corresponding water utility should: perform more detection assays, and increase its awareness of other water characteristics that result from treatment plant performance (Haas and Rose 1995).

Haas et al. (1996) later determined that based on a annual risk of infection of 1 in 10,000 the corresponding oocyst concentration in potable water would be 0.003 oocysts per 100 L. Therefore assuming that a water treatment plant gives rise to a 3-log removal of oocysts, the influent concentration would be 3 oocysts per 100 L for an "acceptable" risk. They further propose that these concentrations should be used to judge a series of samples, not a single sample.

In summary, the health risk of consuming tap water containing C. *parvum* is uncertain. This is in part due to the deficiencies of current laboratory methodologies that often have problems detecting oocysts and further in determining if the protozoa are viable or infectious to humans (Centers for Disease Control and Prevention 1995). Also, the oocyst measurements derived from a detection assay may not be indicative of the dose ingested by the infected individual because of the inherently low recovery of oocysts from water sampling and because of the extreme variability of oocyst distribution in water. Lastly, the infectivity and morbidity of each strain of *Cryptosporidium* spp. within each locale may vary (Haas and Rose 1994) as would the health and the state of the immune system of each exposed individual. In addition, a flaw in current frameworks for action level recommendations is that they do not appear to include the interests of individuals who are immunocompromised. Because these factors may greatly affect the determination of dose-response relationships, further research is required in both the detection and dose response fields. Fewtrell et al. (2001) reviewed the uncertainties in knowledge about waterborne risks as they relate to *Cryptosporidium* spp. and translated them into a "disciplined framework."

5.2.4.2 G. lamblia

The median infectious dose of G. *lamblia* in humans is between 25 and 100 cysts, however, as few as 10 cysts may cause gastroenteritis symptoms (Smith et al. 1995).

A widely used study by Regli et al. (1991) suggests that potable water guidelines should delineate that no more than one *Giardia* spp. or enteric virus infection in 10,000 people per year should occur. Assuming the acceptable annual risk of 10^{-4} for treated water, the acceptable mean concentration for *Giardia* spp. would be 6.75×10^{-7}/L in treated waters (Regli et al. 1991).

Using a 1 in 10,000 annual risk of infection, a single-hit exponential model and a beta-distributed "infectivity probability" model, Rose and Gerba (1991) determined that *Giardia* spp. levels in potable water should be below 0.2 cysts/100 L, assuming that humans consume 2 L of water per day. The geometric average should be below 10^{-3} organisms/ 100 L.

Wallis et al. (1996) proposed an action level of three to five *Giardia* spp. cysts per 100 L in treated potable water. This number is based on monitoring data from outbreak situations.

As with the health risk of consuming C. *parvum* from tap water, the health risks of consuming G. *lamblia* are controversial. As with C. *parvum* this is likely due to the deficiencies of current laboratory methodologies, assessing the actual dose ingested, and the variability of the infectivity and morbidity of each strain of *Giardia* spp. and the state of the immune system of the exposed individuals. And again, the framework for action level recommendations does not appear to include the interests of individuals who are immunocompromised. Further research is therefore required in determining the health risks and action levels when *G. lamblia* is detected in water supplies.

5.2.5 Water Quality Legislation

Saddle Lake Indian Reserve is under Canadian federal jurisdiction for environmental regulations and is therefore governed by the Canada Health Act although Alberta Environment (AE) regulates a large portion of the Saddle Lake drainage basin that does not include the reserve. AE requires that the physical, chemical, microbial and radiological quality of potable water meet the concentration limits established in the latest edition of the "Guidelines for Canadian Drinking Water Quality," produced by the Canadian Council of Ministers of the Environment (CCME) (Alberta Environment 1997, 1999). Likewise AE recreation water quality guidelines often refer to Canadian guidelines. Therefore Saddle Lake water quality requirements are determined by Canadian water quality guidelines.

The following is an overview of Canadian legislation pertinent to Saddle Lake Indian Reserve. To compare with Canadian environmental law, United States Environmental Protection Agency (USEPA) and United Kingdom (UK) standards have been included in the protozoa section.

5.2.5.1 Potable Water

Protozoa

Although Health and Welfare Canada suggests that no viable protozoa be detected within finished waters, no maximum allowable concentrations (MAC) have been developed for protozoa at this time (Health Canada 1999). In 1997, a report entitled "Protozoa in Drinking Water" (Health Canada 1997) suggested a drinking water MAC for *Giardia* spp. and *Cryptosporidium* spp. in drinking water of zero viable organisms detectable per 1000 L. However, it later states that monitoring is not recommended at this time (1997) due to inadequate detection methods and viability and infectivity assays.

Likewise, the USEPA does not have enforceable legislation regarding protozoa, although it does recommend a goal of zero detections of both *G. lamblia* and *Cryptosporidium* spp.[16] A detailed summary of American water quality laws and waterborne pathogens, particularly with regard to *Cryptosporidium* spp. is found in Gostin et al. (2000).

In contrast, new regulations in the United Kingdom (UK) set a water treatment standard of an average of less than one *Cryptosporidium* spp. oocyst in 10 litres of water supplied from water works based on continuous monitoring of a minimum of 40 litres of treated water per hour. In the UK it is a criminal offence for a water company to breach the treatment standard. The UK considers that as the cyst stage of *Giardia* spp. is larger than that of *Cryptosporidium* spp. oocyst, any treatment to remove or reduce *Cryptosporidium* spp. oocysts would also likely be effective for *Giardia* spp.[17]

Coliforms

"The maximum acceptable concentration (MAC) for coliforms in potable water is zero organisms detectable per 100 mL" (Health Canada 1999). However, as they are not uniformly distributed in water and their enumeration is variable, these rules apply:

1. "No sample should contain more than 10 total coliform organisms per 100 mL, none of which should be faecal coliforms;
2. No consecutive sample from the same site should show the presence of coliform organisms; and
3. For community drinking water supplies:
    a) Not more than one sample from a set of samples taken from the community on a given day should show the presence of coliform organisms; and
    b) Not more than 10 % of the samples based on a minimum of 10 samples should show the presence of coliform organisms."

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[16] The USEPA has set the MCLG for *Cryptosporidium* at the genus level due to their conclusion that there is much uncertainty regarding *Cryptosporidium* taxonomy, cross reactions and cross transmission.

[17] Information from correspondence (September 26, 2001) with Peter Jiggins of the Drinking Water Inspectorate (DWI).

Potable water should be re-sampled if the sample contains either more than 500 heterotrophic plate count (HPC) colonies per millilitre, or more than 200 background colonies on a total coliform membrane filter, or if up to 10 total coliform organisms per 100 mL are detected (Health Canada 1999).

Turbidity and pH
According to the Guidelines for Canadian Drinking Water Quality, pH should range between 6.5 and 8.5 and turbidity has an MAC of 1 NTU (nephelometric turbidity unit) and an aesthetic objective (AO) of less than or equal to 5 NTU at the point of consumption (Health Canada 1999).

5.2.5.2 *Recreational Water*

Protozoa
According to the "Recreational Water Quality Guidelines and Aesthetics" (CCME 1999) no limits are specified for pathogenic protozoa. Likewise, no protozoa concentration limits are delineated by AE (Alberta Environment 1999).

Coliforms
According to "Recreational Water Quality Guidelines and Aesthetics," in CCME (1999), no limits have been set for indicator organisms such as coliforms.

AE guidelines state that recreational waters must be withdrawn for treatment prior to distribution as potable water. They indicate "at least 90 percent of the samples (not less than five samples in any consecutive 30-day period) should have a total coliform count of less than 5000 organisms per 100 mL and a faecal coliform count of less than 1000 organisms per 100 mL" (Alberta Environment 1999).

"For direct contact recreation, the geometric mean of not less than five samples taken over not more than a 30-day period should not exceed 1000 organisms per 100 mL for total coliforms, nor 200 organisms per 100 mL for faecal coliforms, nor exceed these numbers in more than 20 percent of the samples examined during any month, nor exceed 2400 organisms per 100 ml total coliforms on any day" (Alberta Environment 1999).

AE also states that with regard to faecal coliforms a geometric mean taken from at least five samples during a period within 30 days should not exceed 200 *E. coli* per 100 mL. When any sample exceeds 400 *E.*

coli per 100 mL re-sampling should occur (Alberta Environment 1999).

Turbidity and pH
The pH range for recreation water should be between 6.5 and 9 (CCME 1999). According to AE pH should be between 6.5 and 8.5 when the buffering capacity of water is low although between 5 and 9 is acceptable (Alberta Environment 1999).

The CCME does not propose turbidity concentrations at this time due to insufficient data (CCME 1999). AE states that turbidity should not be increased more than 5 NTU over natural turbidity when turbidity is less than 50 NTU (Alberta Environment 1999).

5.2.6 Water Management

Although it is essential to optimize water treatment plant processes to provide clean drinking water, controlling pathogen entry to a watershed is the first, and perhaps the most cost-effective, form of reducing the risk of contamination of finished waters (Zhang et al. 1999). Below is a literature review of non-point source and point source management as they relate to *Cryptosporidium* spp. and *Giardia* spp. Specifically it will examine (oo)cyst survival and transport, watershed management, and water and wastewater management primarily as they relate to protozoa. Finally this section will include a brief segment on water treatment studies that have examined (oo)cysts reduction in water treatment plants, focussing on the processes similar to those used at the Saddle Lake water treatment plant.

5.2.6.1 (Oo)cyst Survival

Cryptosporidium spp. oocysts and *Giardia* spp. cysts are fairly resistant to stresses allowing them to survive through unfavourable conditions in faeces, soil and water for long periods of time. The following summarizes a few studies found on the effects of temperature and pH to exemplify the environmental resilience of (oo)cysts.

C. parvum
At temperatures of –4 and 4°C, oocysts can survive in both water and soil for more than 12 weeks while at 25°C oocyst degradation increases according to propidium iodide and mouse infectivity assays (Olson et al. 1999). Some oocysts may be able to withstand a freezing temperature of -22°C (Robertson et al. 1992).

Oocysts are inactivated more rapidly in faeces and soil, which is likely due to the presence of other naturally occurring microorganisms (Olson et al. 1999). Jenkins et al. (1997) found that oocysts inactivation rates when stored in faecal pools in the dark at 4°C for 410 and 259 days is estimated at 0.0040 and 0.0056 oocysts per day, respectively. These results were obtained by dye permeability assays and similar results (0.0046 and 0.0056 oocyst per day) were obtained by excystation assays (Jenkins et al. 1997).

Desiccation may be lethal to *Cryptosporidium* spp. however, oocysts are able to survive in seawater. Contact with faeces may induce enhanced impermeability to small molecules that in turn may increase the protective coat for further exposures to environmental stress (Robertson et al. 1992).

Free ammonia may also increase the inactivation of C. *parvum* oocysts as determined by dye permeability and *in vitro* excystation assays (Jenkins et al. 1998).

G. lamblia

A study by Olson et al. (1999) found that through mouse infectivity and propidium iodide dye exclusion assays *Giardia* spp. cysts were non-infective in water, faeces, and soil after 1 week of freezing at –4°C and within 2 weeks at 25°C. At 4°C *Giardia* spp. cysts were infective for 11 weeks in water, 7 weeks in soil, and 1 week in cattle feces.

Naik et al. (1982) found that 95 percent of *Giardia* spp. cysts were viable at 4°C for up to 90 days. Within 10 days at 0°C cysts were killed. All cysts were killed within 30 min at 70°C. Fifty percent of cysts were killed after storage at 37°C for 7 days.

Naik et al. (1982) also examined the effects of pH on cysts using a trypan blue assay. Eighty to 90 percent of *Giardia* spp. cysts were non-viable at pH 2, 4, 8, and 10 within 5 hours. At pH 6 and 5.6 only 10 to 12 percent of the cysts became non-viable. Viability in tap water was similar to viability in normal saline solution (Naik et al. 1982).

5.2.6.2 Protozoa Transport

Mawdsley et al. (1995) produced a review of published literature on the movement of microbes through soil. According to the review, the following factors influence microbial movement through soil: soil type, soil water content and water flow, surface properties of the microbe, soil pH, plants, temperature, active mobility of the microbe, and micro-

and mesofaunal activity. Microbes move either horizontally or vertically depending on the above factors.

Due to the absorptive properties of its colloidal material, soil type influences microbial transport within soils. Organic matter and clay particles greatly affect the movement of microbial organisms due to microbial adsorption to their negatively charged surfaces. This appears to be more important with the retention of viruses in soil whereas protozoa are more influenced by filtration and sedimentation due to their size. Generally greater protozoa movement occurs in a soil that contains larger pore sizes.[18] Increased water flow rates from precipitation and irrigation also increase the extent of microbial movement (Mawdsley et al. 1995). Darnault et al. (2001) propose that *Cryptosporidium* spp. oocysts may cause a significant hazard of groundwater contamination in a variety of situations due to their ability to penetrate through soil during with augmented rainfall and irrigation.

Horizontal movement may be especially important on grazed lands, feedlots, and land treated with animal manure, slurry, or sewage (Mawdsley et al. 1995). Factors influencing the degree of contamination in horizontal flow (or runoff) include protozoa die-off, water volume, and the sort of waste applied to the fields, topography, water quality, and the extent of water infiltration.

5.2.6.3 Watershed Management Studies

Evidence suggests that (oo)cyst concentrations in surface waters may be linked with watershed use by wildlife such as deer, geese and deer mice (Mager et al. 1998), beavers, aplodonta, otter, marmot (Ongerth et al. 1995), waterfowl (Graczyk et al. 1996), dairy, and beef cattle (Pohjola et al. 1986, Hansen and Ongerth 1991, Ong et al. 1996, States et al. 1997, Zhang et al. 1999, Sischo et al. 2000), human recreational use (Ongerth et al. 1995), and sewage discharge (Wallis et al. 1996, States et al. 1997, Ho and Tam 1998). Strong watershed management appears to influence (oo)cyst presence in drainage basin waters significantly (Hansen and Ongerth 1991).

While point sources such as lagoons and wastewater treatment plant outflows are easier to define, contain, and possibly treat, non-point sources of contaminants are often more difficult to manage. Reducing non-point source contaminants involves three strategies: "reducing or controlling the source of contaminants, collecting and attenuating the delivery of pollutants to receiving waters, and treating contaminated runoff" (Novotny and Chesters, (1981) in Walker et al. (1998)). Major non-point sources of *Giardia* spp. and *Cryptosporidium* spp. are domestic and wild animals although this section will focus only on domestic animals.

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[18] However, protozoa have been found in groundwater. Hancock et al. found protozoa in twelve percent of 199 groundwater sites across the United States (Hancock et al. 1993). A review of the control of microbial pathogens within groundwater can be found in Macler and Merkle (2000).

Reducing or controlling sources of protozoa begins with maintaining domestic animal health. This involves vaccinating young animals and using drug therapies to minimize the intensity and duration of infection and sanitizing livestock housing. While vaccination treatments do exist for *C. parvum*, no drug therapies have been approved for use on animals to stop or prevent infection (Rings and Rings (1996) in Walker et al. (1998)). No sanitation methods have yet been delineated for cleaning interior animal housing areas in part due to the resistance of oocysts to many available disinfectants (Blewett (1989) and Campbell et al. (1982) in Walker et al. (1998)).

Manure management may be essential in decreasing (oo)cyst runoff into streams and rivers (Zhang et al. 1999, Sischo et al. 2000). Olsen et al. (1999) recommend storing manure for 12 weeks prior to distributing cattle feces (during warmer weather) on fields. Piles of farmyard manure can rise to temperatures of 70°C (Jones 1980), which can kill cysts (Naik et al. 1982); therefore stored manure can greatly reduce the number of pathogens that can potentially contaminate water supplies.

Note that young calves are perhaps the largest contributor to oocyst environmental contamination as they have a high positive rate and high shedding rates. Therefore the management of neonatal calves, especially during calving periods and during high levels of precipitation, may be very important for watershed management (Zhang et al. 1999).

Attenuating the delivery of pollutants to receiving waters can be achieved in a variety of ways. Traditionally livestock are bedded on large amounts of straw and the waste is composted and utilized as fertilizer. As mentioned previously, Jones (1980) suggests that composted manure can reach temperatures as high as 70°C by aerobic processes, thereby destroying the majority of pathogens within. However, with more intensive livestock operations, waste is now more often being collected as slurry (semi-liquid form). These wastes are collected and stored in what becomes an anaerobic condition and the reduction of pathogens, which occurs in composted manure, does not occur (Rankin and Taylor 1969). According to

Mawdsley et al. (1995), wastes spread on fields without composting not only has the potential for water pollution and hence human infections, but it also increases the risk of livestock infections. The health risks for livestock and humans are also increased by large amounts of soiled water derived from sources such as milking parlours and animal house cleaning wastes, which are often used to irrigate grasslands (Mawdsley et al. 1995).

The risk of contaminating a water supply depends on the parameters of the watershed area such as: slope, vegetation, wildlife (and domestic animal) concentration and proximity to water bodies (James et al. 1994); soil types, due to their relative porosities; weather (e.g. runoff and temperature variations); geology; propensity for stream bank erosion; and size and composition of riparian buffers (Sovell et al. 2000). As such, watershed management often involves land management around watercourses. Although protozoa may or may not act as sediment[19] (Walker et al. 1998), one method of attenuating (oo)cysts within runoff is to equate (oo)cysts with sediment and simply attempt to decrease erosion in stream or river banks such as near grazing areas.

Sovell et al. (2000) examined the effects of rotational and continuous grazing pastures and a variety of riparian buffer strips on: water chemistry, physical habitat, benthic macroinvertebrates, and fish, all as indicators of stream quality. They found higher faecal coliform and turbidity results at continuously grazed sites as opposed to rotational grazed sites.

A study by Daniels and Gilliam (1996) found that runoff loads were reduced by 50 to 80 percent from North Carolina agricultural fields with grass and riparian filter strips. Total sediment was decreased by about 80 percent.

5.2.6.4 Wastewater Treatment

Point sources of (oo)cysts such as wastewater treatment plants and lagoons may be managed to provide the least amount of environmental contamination by a variety of means. The following outlines the few studies found on (oo)cyst management that pertain to smaller plants or lagoons, as found within the Saddle Lake watershed. Clearly, more research is required in this area.

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[19] Oocysts appear to have a negative charge and may therefore be unlikely to adsorb to negatively charged clay minerals and hydrophobic organic matter.

Increased potential for environmental contamination occurs when effluent from wastewater treatment plants (and therefore wastewater lagoons) is released during periods of dry weather due to higher concentrations of the pathogens in lower volumes of water (Zhang et al. 1999). Therefore effluent release may be managed according to weather and water levels.

Artificial wetlands may be designed to reduce large numbers of (oo)cysts. A study of artificial wetland design for the removal of protozoa in secondary wastewater examined three wastewater designs: a duckweed-covered pond, a multi-species subsurface flow, and a multi-species surface flow wetland. *Giardia* spp. and *Cryptosporidium* spp. removal was the highest in the duckweed pond at 98 and 89 percent removals, respectively. *Giardia* spp. concentrations were reduced from the range of 4 to 33 cysts per L to 0 to 1 cysts per L from the influent to the effluent range. Similarly, *Cryptosporidium* spp. oocysts decreased from the range of 0 to 3 oocysts per L to 0 to 1 oocysts per L from the influent to the effluent range in the duckweed system. The least amount of removal occurred in the multi-species surface flow wetland at 73 and 58 percent for *Giardia* spp. and *Cryptosporidium* spp., respectively. This study suggests that a combination of wetland and subsurface flow strategies may give rise to the highest level of treatment (Gerba et al. 1999).

5.2.6.5 Potable Water Treatment

Potable water treatment is the final contaminant barrier to protect human health. The following section summarizes several studies pertaining to commonly found microbial reduction methods, such as those found at Saddle Lake water treatment plant, and their efficacy as they relate to protozoa. Note that microbial reduction studies use variable detection and assay methods and therefore are generally not comparable. Detection recoveries are often not documented and therefore results can be difficult to interpret.

A study by States et al. (1997) of the Pittsburgh, Pennsylvania water treatment plant, suggests that their conventional clarification and filtration appears to completely eliminate *Giardia* spp. cysts in the treated water supply although they found that small numbers of *Cryptosporidium* oocysts might occasionally pass through their treatment facility. Their utility treats approximately 0.25 to 0.26 x 10^6 m^3/d by clarification using ferric chloride coagulation, flocculation, and settling in a small primary basin and a 0.45 x 10^6 m^3 secondary sedimentation basin. The water then passes through a dual-media rapid sand filter of anthracite coal, sand and support gravel and free chlorine microbial reduction.

Filter backwash waters from the plant contained high numbers of *Giardia* spp. and *Cryptosporidium* spp., suggesting that backwash recycle waters could be a large source of contamination in treatment plant intake waters (States et al. 1997, Karanis et al. 1998). Karanis et al. (1998) suggest that backwash recycling should not occur without specialized backwash water filtration or disinfection treatment. Karanis et al. (1998) also found that the combination of slow sand filtration, infiltration, disinfection, sand and activated carbon filtration was an effective water treatment system for reducing (oo)cyst concentrations.

Dumoutier and Mandra (1996) recommend optimizing the clarification process to remove (oo)cysts. They also suggest an ozonation step, which would improve (oo)cyst removal, however, they state that membrane filtration should be used as a polishing treatment as they believe that it is the "best available technology for cyst removal."

Edzwald and Kelley (1998) indicate that coagulation, clarification, and filtration are critical for the control of *Cryptosporidium* spp. Dissolved air flotation can give rise to 3 log removals of oocysts compared to a 1-log removal by sedimentation. Dissolved air flotation and filtration combined can give rise to a cumulative log removal of 4 to 5.

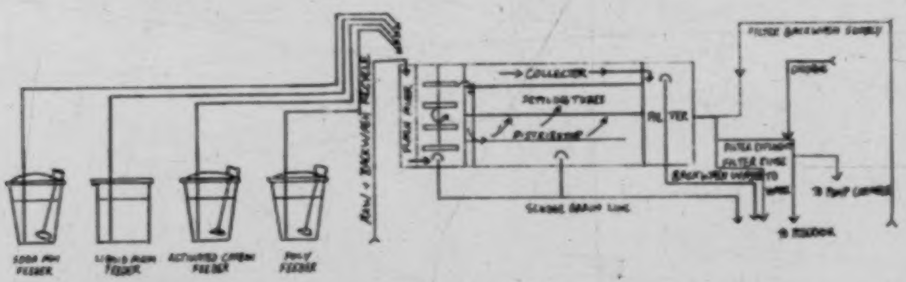
A study on *Giardia* spp. and *Cryptosporidium* spp. removal on both a 1.9 L/min pilot plant and a 3407 L/min water treatment plant operating under conventional treatment and direct filtration regimes indicated that the removals of *Giardia* spp. were consistently greater than the removals of *Cryptosporidium* spp. *Giardia* spp. removals were between 2.2 and 4 log compared with 1.9 to 3 log for *Cryptosporidium* spp. They also found that results for (oo)cyst removal in conventional treatment was comparable to direct filtration results (Nieminski and Ongerth 1995). They conclude that "In a properly operating treatment plant producing finished water turbidity of 0.1 to 0.2 NTU, either conventional treatment or direct filtration can achieve a 3-log removal of *Giardia* spp. cysts" and that *Cryptosporidium* spp. oocysts are more difficult to remove (with a 0.4 log difference).

There are a multitude of studies that have examined *Giardia* spp. and *Cryptosporidium* spp. microbial reduction with UV (Clancy et al. 1998, Craik et al. 2001), ozone (Gyurek et al. 1999, Li et al. 2001), and chlorine (Finch et al. 1995, Liyanage 1998).

5.2.7 Saddle Lake Water Treatment System and Process Flow

Saddle Lake water intake is 133 m from the pump station and 1 m above the bottom of the lake. It is initially drawn through a screen on the end of the lake intake pipe that has 1 and 2 mm openings. The water proceeds through the pump house where it is presently mixed with recycled backwash waste from the filter backwash re-circulation ponds (GPEC Consulting Ltd. 1994). The lake water turbidity recorded at the water treatment plant ranges between less than 2 NTU during the winter to 7 NTU during the summer (Scott 2000).

The mixture of raw and recycled backwash water is treated by two package water treatment plants through flocculation, coagulation, filtration and chlorination (Robb 2000). Figure 2, adapted from GPEC Consulting Ltd. (1994) illustrates the process flow of both package treatment plants.



• Figure 2: Saddle Lake water treatment plant layout. Figure adapted from GPEC Consulting Ltd. (1994).

The two package water treatment plants combined are designed to operate at a maximum of 15.2 L/s (200 Imperial gallons per minute), which complies with standard detention times and filtration rates. According to the plant operators, optimum water quality is produced at 14.0 L/s (185 Imperial gallons per minute) (Laidlaw 1999b).

Potable water is distributed via two rural distribution systems and water trucks.

5.2.7.1 Design criteria for the 1994 upgrade

- Population: 3100
- 186 L/ person/ day (41 imperial gallons per day (Igpd))

- Average Daily Flow: 577,340 L/day (127,000 gallons/ day (Igpd)) (17,320,200 L/month)
- Peak Daily Flow: 832 L/s (177 gallons/ minute (Igpm))
- Plant A Capacity: 15.1 L/s (200 Igpm)
- Plant B Capacity: 5.3 L/s (70 Igpm)
- Raw Water Pump Rate: 15.8 L/s (208 Igpm)
- Transfer Pump Rate: 15.8 L/s (208 Igpm)
- Filter Backwash Rate: 40.7 L/s (540 Igpm) (Laidlaw 1999b)

5.2.7.2 Present Operations

- Delivered Water (average): 18,184,360 L/month (4,000,000 Imperial gallons per month(Ig/month))
- Backwashing: 7,728,353 L/month (1,700,000 Ig/month (average)
- Total production: 25,912,713 L/month (5,700,000 Ig/month) (average)
- Average Daily Consumption: 10,001,398 L/d (220,000 Igpd)
- Peak Daily Demand: 1388 L/min (305.4 Igpm)
- Present Population: approximately 5,000 (6,000 on member list)
- Per Capita Consumption: 200 L/d (44 Igpd)
- Present Plant Capacity for optimum water quality: 14 L/s (185 Igm) (Laidlaw 1999a)

5.2.7.3 Performance

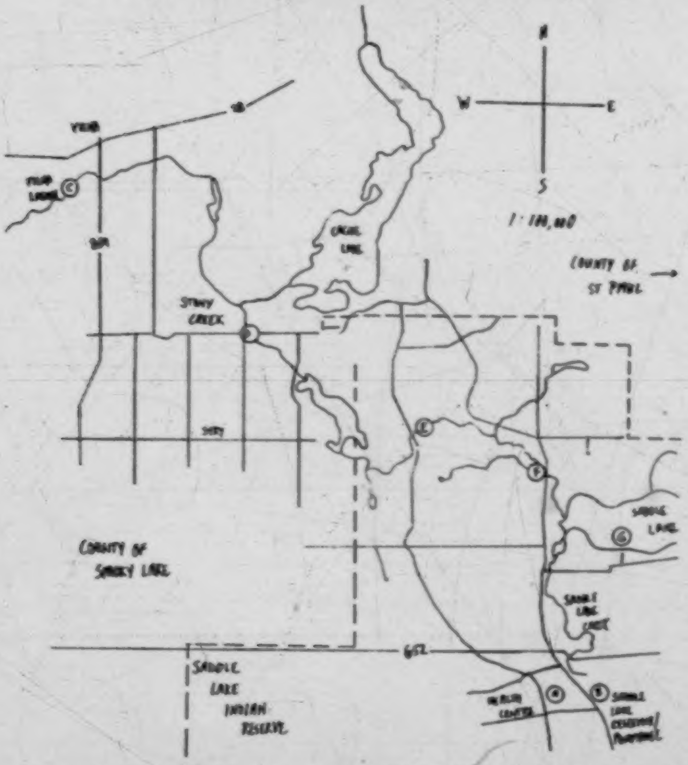
The Saddle Lake water treatment plant complies with the Alberta Environmental (AE) guidelines for rapid sand filtration plants. According to AE, turbidity reduction should be 80 percent when turbidities are less than 2.5 NTU. Turbidity should be less than 0.5 NTU when initial turbidity is greater than 2.5 NTU. In addition this means that the water treatment plant achieves 2.5 log (99.7 percent) credit for *Giardia* spp. reduction and 2.0 log (99 percent) removal credit for virus reduction. In 2000, GPEC Consulting showed that the Saddle Lake plant exceeded the AE minimum requirement by achieving a 5.5 log credit for *Giardia* spp. removal and a 6.0 log credit for virus removal from combined filtration and chlorination treatment (Scott 2000).

Although Canadian waters are not under its jurisdiction, according to the Safe Drinking Water Act (United States Public Health Services), "a plant that achieves consistent filter effluent turbidity of 0.3 NTU would receive a 2 log credit for *Cryptosporidium* spp. reduction (99 percent). Saddle Lake consistently achieves between 0.1 and 0.2 NTU filter effluent which, combined with other parameters, would indicate a 3 log (99.9 percent reduction) removal credit is probably available" (Scott 2000).

5.3 EXPERIMENTAL DESIGN

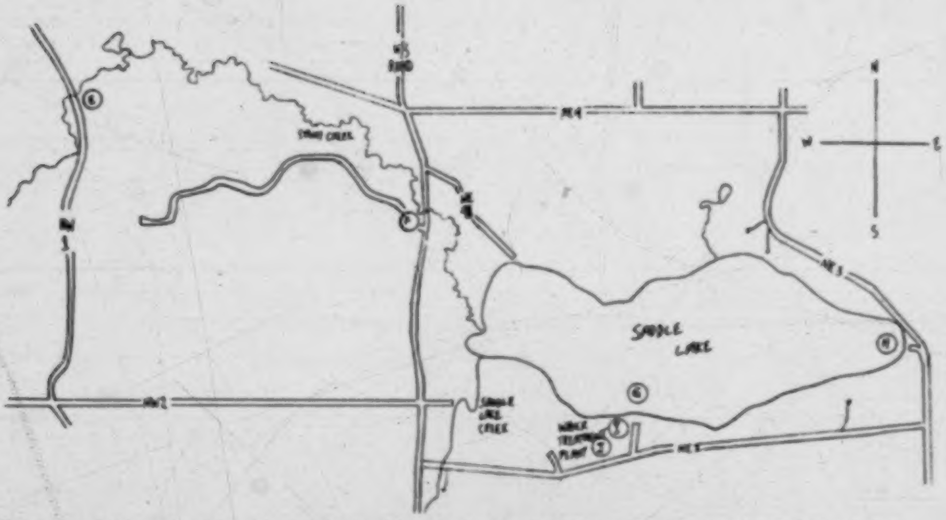
5.3.1 Sampling Locations

Water sampling was performed from November 2000 to August 2001. See Figure 3 for an overview of the sampling locations along Stony Creek (Land Data Technologies Inc. 1997). Figure 4 depicts the sampling locations near Saddle Lake (Torchinsky Engineering Ltd. 1992) and Figure 5 depicts the sampling locations at the Saddle Lake water treatment plant (GPEC Consulting Ltd. 1994).

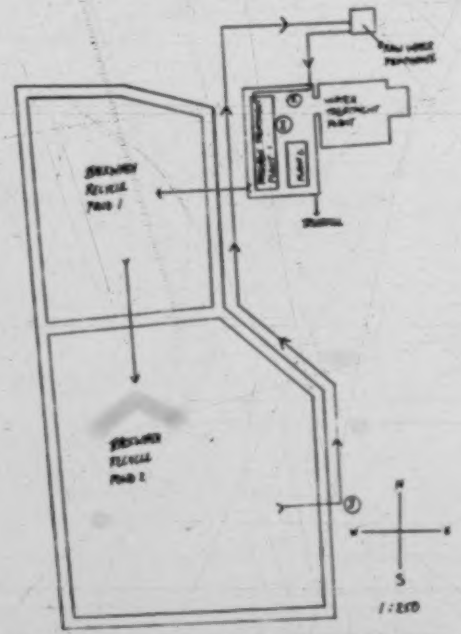


VILNA
36
VILNA LAGOON
N
W
E
S
1:100,000
COUNTY OF ST PAUL
STONY CREEK
COUNTY OF SMOKY LAKE
SADDLE LAKE
SADDLE LAKE CREEK
652
SADDLE LAKE INDIAN RESERVE
HEALTH CENTRE
SADDLE LAKE RESERVOIR/ PUMPHOUSE

• Figure 3: Sampling locations along Stony Creek from the town of Vilna's lagoon to Saddle Lake and potable water sampling locations at the Health Centre and the Saddle Lake reservoir/ pump house. Figure adapted from Land Data Technologies Inc. (1997).


N
W
E
S
STONY CREEK
SADDLE LAKE
SADDLE LAKE CREEK
WATER TREATMENT PLANT

• Figure 4: Sampling locations around Saddle Lake and Stony Creek on the Saddle Lake Reserve. Figure adapted from Torchinsky Engineering Ltd (1992).


RAW WATER PUMPHOUSE
WATER TREATMENT PLANT
BACKWASH RECYCLE POND 1
BACKWASH RECYCLE POND 2
N
W
E
S
1:250

• Figure 5: Saddle Lake water treatment plant layout and sampling locations. Figure adapted from GPEC Consulting Ltd. (1994).

Table 8 correlates the letters on the previous maps with the locations on the tables within the results and discussion section.

- Table 8: Sample locations as they relate to descriptions in results section.

Sample Location According to Maps	Sample Location	Location Specifics	Potable/ Raw
A	Health Centre	Garage or boiler room	Potable
B	Reservoir		Potable
C	Vilna Lagoon	Effluent	Raw
D	Stony Creek	D	Raw
E	Stony Creek	E	Raw
F	Stony Creek	F	Raw
G	Saddle Lake	Below pump house	Raw
H	Saddle Lake	Recreational area	Raw
I	Water Treatment Plant		Potable
J	Backwash pond	Recycle	Raw
K	Water treatment plant	Raw & backwash	Raw

5.4 RESULTS

Table 9 summarizes infectivity and FACS-sorted microscope count results. No infections were caused by *C. parvum* in the mice. Two treated water samples, 10 and 11, caused giardiasis in 2 of 4 and 1 of 4 gerbils, respectively.

Tables 10 to 13 summarize flow cytometer and FACS detection results. The single gates, R3 for *Giardia* spp. and R1 for *Cryptosporidium* spp., represented the number of particles that were similar to the protozoa in size and internal complexity. It is assumed that these single regions were correlated with proportion of the sample used in the initial data file and the proportion of the sample that was sorted. Therefore the single gates were used to determine the proportion of sample that was sorted and consequently the volume of water filtered. The double gates were counts of particles actually sorted by the FACS and were not used in calculations.

The following applies to Tables 10 to 13:

Volume of filtered water assessed by sorting (L) =

Total volume filtered (L)/ 4 * (sorted sample R3/ (initial data file sample R3 + sorted sample R3))

Therefore the concentration of (oo)cysts per L was determined by the ratio of actual (oo)cyst counts over the volume of filtered water assessed by sorting. The total volume filtered was initially divided by four as each concentrate was used for four different analyses.[20]

According to the samples of potable water in Table 10, 1 of 8 health centre samples was positive with a concentration of 0.972 cysts/L. Two of 7 water treatment plant potable water samples were positive with concentrations of 0.004 and 0.041 cysts/L. Health centre and water treatment plant samples 10 and 11, with concentrations of 0.972 and 0.041 cysts/L, gave rise to giardiasis in gerbils. The one sample taken from the reservoir was negative for *Giardia* spp. cysts.

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[20] Except for samples 33 to 37 which were not assessed by infectivity and were therefore only split into two sub-samples.

Table 11 indicates that 2 of 8 health centre samples were positive with concentrations of 0.004 and 1.623 *Cryptosporidium* spp. oocysts/L. Note that samples 29 and 30 were sampled on the same day. While sample 29 had a concentration of 1.623 oocysts/L, the latter was negative, thus producing results that are difficult to explain. One of 7 water treatment plant potable water samples was positive with a concentration of 0.026 oocysts/L. The one Saddle Lake reservoir sample was positive with a concentration of 0.004 oocysts/L.

As per Table 12, *Giardia* spp. detections in raw waters were few. One sample from each of the water treatment plant intake, Stony Creek and Vilna lagoon were positive with concentrations of 0.129, 0.222, and 0.484 cysts/L, respectively. All other raw water samples assessed for *Giardia* spp. were negative, including the other 7 water treatment plant intake samples, 5 other Stony Creek samples, 1 Saddle Lake recreation area sample and 1 backwash pond recycle sample.

Table 13 summarizes *Cryptosporidium* spp. oocyst detections in raw water. Three of 8 water treatment plant intake samples were positive for oocysts with concentrations of 0.197, 0.088 and 0.041 oocysts/L. One of 2 Saddle Lake samples was positive with a concentration of 0.241 oocysts/L in the recreation area. One of 7 Stony Creek samples was positive with a concentration of 0.082 oocysts/L. Neither of the Vilna lagoon samples nor the backwash pond recycle sample was positive for *Cryptosporidium* spp. oocysts.

A summary of all Saddle Lake water sampling data is provided in Table 14, including total coliform, faecal coliform, heterotrophic plate count, turbidity and pH water quality parameters. No correlations were found by linear regression between oocyst and cyst concentrations nor between (oo)cyst concentrations and any other parameter.

• Table 9: Summary of *Giardia* spp. and *Cryptosporidium* spp. infectivity and detection results from potable and raw water within the Saddle Lake Drainage Basin. See tables 10 and 13 for concentrations of (oo)cysts per litre of filtered water derived from flow cytometer.

							Cryptosporidium		*Giardia*	
Season	Sample	Source	Potable/ Raw	Location Specifics	Sampling Date	Total Volume Filtered (L)	Mouse Infections	Detected Oocysts	Gerbil Infections	Detected Cysts
AUTUMN	1	Health Centre	Potable	Garage	20-Nov-00	1000	0/10	0	0/4	0
	2	Saddle Lake	Raw	Recreation area	20-Nov-00	50	0/10	2	0/4	0
	3	Water Treatment Plant	Raw	Raw & backwash	21-Nov-01	50	0/10	2	0/4	0
	4	Stony Creek	Raw	D	21-Nov-01	50	0/10	1	0/4	0
	5	Stony Creek	Raw	E	21-Nov-01	36	0/10	0	0/4	0
	6	Stony Creek	Raw	F	21-Nov-01	50	0/10	0	0/4	0
WINTER	7	Health Centre	Potable	Garage	30-Jan-01	1071	0/10	0	-	-
	8	Water Treatment Plant	Potable		30-Jan-01	1010	0/10	0	-	0
	9	Water Treatment Plant	Raw	Raw & backwash	30-Jan-01	50	0/10	1	-	1
	10	Health Centre	Potable	Garage	22-Feb-01	1181	0/10	0	2/4	28
	11	Water Treatment Plant	Potable		22-Feb-01	1000	0/10	0	1/4	3
	12	Stony Creek	Raw	F	22-Feb-01	50	0/10	0	0/4	0
	13	Water Treatment Plant	Raw	Raw & backwash	22-Feb-01	50	0/10	0	0/4	0
SPRING	14	Health Centre	Potable	Garage	05-Apr-01	1004	0/10	0	0/4	0
	15	Water Treatment Plant	Potable		05-Apr-01	1410	0/10	0	0/4	1
	16	Water Treatment Plant	Raw	Raw & backwash	05-Apr-01	50	0/10	0	0/4	0
	17	Saddle Lake	Raw	Below pumphouse	05-Apr-01	50	0/10	0	-	-
	18	Stony Creek	Raw	F	05-Apr-01	50	0/10	0	0/4	2
	19	Health Centre	Potable	Garage	23-Apr-01	1014	0/10	0	0/4	0
	20	Water Treatment Plant	Potable		23-Apr-01	1019	0/10	0	0/4	0
	21	Water Treatment Plant	Raw	Raw & backwash	23-Apr-01	50	0/10	0	0/4	0
	22	Backwash Pond	Raw	Backwash recycle	23-Apr-01	50	0/10	0	0/4	0
	23	Health Centre	Potable	Garage	07-May-01	1000	0/10	0	0/4	0
	24	Water Treatment Plant	Potable		07-May-01	1069	0/10	0	0/4	0
	25	Vima Lagoon Effluent	Raw	Near Surface	07-May-01	50	0/10	0	0/4	2
	26	Vima Lagoon Effluent	Raw	Near Bottom	07-May-01	50	0/10	0	-	-
	27	Stony Creek	Raw	F	07-May-01	50	0/10	0	-	-
	28	Water Treatment Plant	Raw	Raw & backwash	07-May-01	50	0/10	0	0/4	0
SUMMER	29	Health Centre	Potable	Garage	16-Jul-01	1020.5	0/10	300	0/4	0
	30	Water Treatment Plant	Potable		16-Jul-01	1153.6	0/10	0	0/4	0
	31	Water Treatment Plant	Raw	Raw & backwash	16-Jul-01	50	0/10	0	0/4	0
	32	Stony Creek	Raw	F	16-Jul-01	50	0/10	0	0/4	0
	33	Health Centre	Potable	Boiler room (See37)	03-Aug-01	1269	Resampled - See 37		-	0
	34	Water Reservoir	Potable	In townsite	03-Aug-01	1090	-	2	-	0
	35	Water Treatment Plant	Potable		03-Aug-01	1000	-	12	-	0
	36	Water Treatment Plant	Raw	Raw & backwash	03-Aug-01	50	-	1	-	0
	37	Health Centre	Potable	Re-sampled 33	15-Aug-01	1000	-	2	-	0

- Table 10: Summary of flow cytometry and confirmed *Giardia* spp. cyst results from potable water samples.

				Initial data file sample			Sorted sample							
Sample Number	Source	Source Specifics	Date of Sampling	Date of Analysis	Number of Particles in R3	Number of Particles in R3 and R2	Date of Analysis	Number of Particles in R3	Number of Particles in R3 and R2	Actual Cyst Count	Percent of Sample Sorted	Total Volume Filtered (L)	Volume of Filtered Water Assessed by Sorting (L)	Concentration of Cysts/L
1	Health Centre	Garage	19-Nov-00	24-Nov-00	11870	37	24-Nov-00	18205	30	0	61%	1000	151	0.000
8	Water Treatment Plant		29-Jan-01	2-Feb-01	7902	24	2-Feb-01	8308	24	0	51%	1010	129	0.000
10	Health Centre	Garage	21-Feb-01	25-Feb-01	92971	114	26-Feb-01	10047	176	28	10%	1181	29	0.972
11	Water Treatment Plant		21-Feb-01	25-Feb-01	5058	89	26-Feb-01	2121	117	3	30%	1000	74	0.041
14	Health Centre	Garage	04-Apr-01	10-Apr-01	34879	99	10-Apr-01	64216	166	0	65%	1004	163	0.000
15	Water Treatment Plant		04-Apr-01	10-Apr-01	40844	77	10-Apr-01	105700	174	1	72%	1410	254	0.004
19	Health Centre	Garage	22-Apr-01	30-Apr-01	40565	72	30-Apr-01	136405	195	0	77%	1014	195	0.000
20	Water Treatment Plant		22-Apr-01	30-Apr-01	97538	570	30-Apr-01	475014	1558	0	83%	1019	211	0.000
23	Health Centre	Garage	06-May-01	14-May-01	179676	110	15-May-01	91665	127	0	34%	1000	84	0.000
24	Water Treatment Plant		06-May-01	14-May-01	276892	229	15-May-01	349699	2140	0	56%	1069	149	0.000
29	Health Centre	Garage	15-Jul-01	21-Jul-01	60956	1166	21-Jul-01	22215	920	0	27%	1020.5	68	0.000
30	Water Treatment Plant		15-Jul-01	21-Jul-01	93355	10000	21-Jul-01	517667	48331	0	85%	1153.6	244	0.000
33	Health Centre	Boiler room	02-Aug-01	7-Aug-01	44814	204	7-Aug-01	313306	1029	0	87%	1269	555	0.000
34	Water Reservoir	in townsite	02-Aug-01	7-Aug-01	83356	195	7-Aug-01	867613	1536	0	91%	1090	497	0.000
35	Water Treatment Plant		02-Aug-01	7-Aug-01	111344	178	7-Aug-01	633682	1039	0	85%	1000	425	0.000
37	Health Centre	Boiler room	14-Aug-01	17-Aug-01	160885	101	17-Aug-01	1165660	662	0	86%	1000	439	0.000

• Table 11: Flow cytometry and confirmed *Cryptosporidium* spp. oocyst results from potable water samples.

				Initial Data File Sample			Sorted Sample							
Sample Number	Source	Source Specifics	Sampling Date	Date of Analysis	Number of Particles in R1	Number of Particles in R1 and R2	Date of Analysis	Number of Particles in R1	Number of Particles in R1 and R2	Actual Oocyst Count	Percent of Sample Sorted	Total Volume Filtered (L)	Volume of Filtered Water Assessed by Sorting (L)	Concentration of Oocysts/L
1	Health Centre	Garage	19-Nov-00	26-Nov-00	3225	5	27-Nov-00	8097	12	0	72%	1000	179	0.000
7	Health Centre	Garage	29-Jan-01	2-Feb-01	48364	9	2-Feb-01	155247	74	0	77%	1071	207	0.000
8	Water Treatment Plant		29-Jan-01	2-Feb-01	64527	13	3-Feb-01	183421	33	0	74%	1010	187	0.000
10	Health Centre	Garage	21-Feb-01	26-Feb-01	19607	3	27-Feb-01	40623	4	0	67%	1181	199	0.000
11	Water Treatment Plant		21-Feb-01	25-Feb-01	12218	3	27-Feb-01	19761	4	0	62%	1000	154	0.000
14	Health Centre	Garage	04-Apr-01	10-Apr-01	77339	2	10-Apr-01	427208	10	0	86%	1004	213	0.000
15	Water Treatment Plant		04-Apr-01	10-Apr-01	73436	0	11-Apr-01	364652	7	0	83%	1410	293	0.000
19	Health Centre	Garage	22-Apr-01	30-Apr-01	84430	5	30-Apr-01	624025	17	0	88%	1014	223	0.000
20	Water Treatment Plant		22-Apr-01	30-Apr-01	157301	27	30-Apr-01	742993	60	0	83%	1019	210	0.000
23	Health Centre	Garage	06-May-01	14-May-01	86308	5	15-May-01	691516	44	0	89%	1000	222	0.000
24	Water Treatment Plant		06-May-01	14-May-01	238451	4	15-May-01	7945	0	0	3%	1089	9	0.000
29	Health Centre	Garage	15-Jul-01	21-Jul-01	188023	242	21-Jul-01	494825	767	300	72%	1020.5	186	1.823
30	Water Treatment Plant		15-Jul-01	21-Jul-01	236268	2	21-Jul-01	1568060	8	0	87%	1153.6	251	0.000
34	Water Reservoir	In townsite	02-Aug-01	7-Aug-01	254909	2	7-Aug-01	3848759	25	2	94%	1080	512	0.004
35	Water Treatment Plant		02-Aug-01	7-Aug-01	273903	22	7-Aug-01	3140537	277	12	92%	1000	460	0.026
37	Health Centre	Boiler Room	14-Aug-01	17-Aug-01	109514	1	17-Aug-01	959219	15	2	90%	1000	449	0.004

• Table 12: Summary of flow cytometer and actual *Giardia* spp. cyst counts from raw water samples.

				Initial data file sample			Sorted sample							
Sample Number	Source	Source Specifics	Date of Sampling	Date of Analysis	Number of Particles in R3	Number of Particles in R3 and R2	Date of Analysis	Number of Particles in R3	Number of Particles in R3 and R2	Actual Cyst Count	Percent of Sample Sorted	Total Volume Filtered (L)	Volume of Filtered Water Assessed by Sorting (L)	Concentration of Cysts/L
2	Saddle Lake	Recreation area	19-Nov-00	24-Nov-00	6536	35	24-Nov-00	18393	112	0	74%	50	9	0.000
3	Water Treatment Plant	Raw & backwash	20-Nov-01	24-Nov-00*	3179	28	24-Nov-00	12146	80	0	79%	50	10	0.000
4	Stony Creek	D	20-Nov-01	24-Nov-00	26754	175	24-Nov-00	188320	712	0	88%	50	11	0.000
5	Stony Creek	E	20-Nov-01	24-Nov-00	20088	3091	26-Nov-00	429	86	0	2%	36	0	0.000
6	Stony Creek	F	20-Nov-01	24-Nov-00	2935	6	26-Nov-00	49	6	0	2%	50	0	0.000
9	Water Treatment Plant	Raw & backwash	29-Jan-01	2-Feb-01	44334	52	2-Feb-01	71939	87	1	62%	50	8	0.129
12	Stony Creek	F	21-Feb-01	25-Feb-01	129844	169	26-Feb-01	26931	96	0	17%	50	2	0.000
13	Water Treatment Plant	Raw & backwash	21-Feb-01	25-Feb-01	128136	66	26-Feb-01	63899	124	0	33%	50	4	0.000
16	Water Treatment Plant	Raw & backwash	04-Apr-01	10-Apr-01	65472	111	10-Apr-01	118069	154	0	64%	50	8	0.000
18	Stony Creek	F	04-Apr-01	10-Apr-01	47299	71	10-Apr-01	121329	159	2	72%	50	9	0.222
21	Water Treatment Plant	Raw & backwash	22-Apr-01	30-Apr-01	44191	9	30-Apr-01	683641	188	0	94%	50	12	0.000
22	Backwash Pond	Backwash recycle	22-Apr-01	30-Apr-01	30939	39	30-Apr-01	79672	115	0	72%	50	9	0.000
25	Vina Lagoon Effluent	Near Surface	08-May-01	14-May-01	521212	113	15-May-01	257618	298	2	33%	50	4	0.484
28	Water Treatment Plant	Raw & backwash	08-May-01	14-May-01	292061	75	15-May-01	338318	138	0	54%	50	7	0.000
31	Water Treatment Plant	Raw & backwash	15-Jul-01	21-Jul-01	93836	134	21-Jul-01	344958	2438	0	79%	50	10	0.000
32	Stony Creek	F	15-Jul-01	21-Jul-01	93836	134	21-Jul-01	383975	417	0	80%	50	10	0.000
36	Water Treatment Plant	Raw & backwash	02-Aug-01	7-Aug-01	197749	374	7-Aug-01	4309186	10660	0	98%	50	24	0.000

• Table 13: Summary of *Cryptosporidium* spp. oocyst detections and flow cytometry counts from Saddle Lake raw water samples.

Sample Number	Source	Source Specifics	Sampling Date	Initial Data File Sample			Sorted Sample			Actual Oocyst Count	Percent of Sample Sorted	Total Volume Filtered (L)	Volume of Filtered Water Assessed by Sorting (L)	Concentration of Oocysts/L
				Date of Analysis	Number of Particles in R1	Number of Particles in R1 and R2	Date of Analysis	Number of Particles in R1	Number of Particles in R1 and R2					
2	Saddle Lake	Recreation area	19-Nov-00	26-Nov-00	1075	24	27-Nov-00	2125	33	2	86%	50	8	0.241
3	Water Treatment Plant	Raw & backwash	20-Nov-01	26-Nov-00	1642	9	28-Nov-00	7152	54	2	81%	50	10	0.197
4	Stony Creek	D	20-Nov-01	26-Nov-00	960	1	29-Nov-00	29292	57	1	96%	50	12	0.082
5	Stony Creek	E	20-Nov-01	26-Nov-00	603	36	29-Nov-00	926	21	0	69%	36	6	0.000
6	Stony Creek	F	20-Nov-01	26-Nov-00	132	4	29-Nov-00	868	11	0	84%	50	10	0.000
9	Water Treatment Plant	Raw & backwash	29-Jan-01	2-Feb-01	183830	70	3-Feb-01	1871476	379	1	91%	50	11	0.088
12	Stony Creek	F	21-Feb-01	25-Feb-01	68839	2	27-Feb-01	229094	3	0	77%	50	10	0.000
13	Water Treatment Plant	Raw & backwash	21-Feb-01	25-Feb-01	196071	44	27-Feb-01	436060	61	0	74%	50	9	0.000
16	Water Treatment Plant	Raw & backwash	04-Apr-01	10-Apr-01	188649	3	11-Apr-01	869413	13	0	62%	50	10	0.000
17	Saddle Lake	Below pumphouse	04-Apr-01	10-Apr-01	155417	3	11-Apr-01	853791	21	0	86%	50	11	0.000
18	Stony Creek	F	04-Apr-01	10-Apr-01	131673	5	11-Apr-01	1163277	62	0	90%	50	11	0.000
21	Water Treatment Plant	Raw & backwash	22-Apr-01	30-Apr-01	99416	5	1-May-01	2221307	24	0	96%	50	12	0.000
22	Backwash Pond	Backwash recycle	22-Apr-01	30-Apr-01	126904	25	1-May-01	939782	111	0	89%	50	11	0.000
25	Vina Lagoon Effluent	Near Surface	06-May-01	14-May-01	320208	5	15-May-01	1219839	51	0	79%	50	10	0.000
26	Vina Lagoon Effluent	Near Bottom	06-May-01	14-May-01	388223	6	16-May-01	1001917	63	0	73%	50	9	0.000
27	Stony Creek	F	06-May-01	14-May-01	52904	0	16-May-01	4521556	2	0	99%	50	12	0.000
28	Water Treatment Plant	Raw & backwash	06-May-01	14-May-01	167913	3	16-May-01	8309370	36	0	98%	50	12	0.000
31	Water Treatment Plant	Raw & backwash	15-Jul-01	21-Jul-01	166121	0	22-Jul-01	3153361	29	0	96%	50	12	0.000
32	Stony Creek	F	15-Jul-01	21-Jul-01	92372	40	22-Jul-01	4553575	318	0	98%	50	12	0.000
36	Water Treatment Plant	Raw & backwash	02-Aug-01	7-Aug-01	188075	3	7-Aug-01	5827271	120	1	97%	50	24	0.041

• Table 14: Summary of all Saddle Lake drainage basin protozoan detection and water quality parameter results. Note that samples 10 and 11 caused giardiasis in a fraction of gerbils. (* Denotes not done. ** Denotes that further tests have confirmed that not all of the organisms previously reported are coliforms; some represent other species of bacteria which are not in the coliform group.)

Season	Sample Number	Source	Potable/ Raw	Location Specifics	Sampling Date	Concentration per L		Count per 100 mL		CFU/mL	NTU	
						Cysts	Oocysts	Total Coliform	Faecal Coliform	Heterotrophic Plate Count	Turbidity	pH
AUTUMN	1	Health Centre	Potable	Garage	20-Nov-00	0	0	0	0	<10	0.2	7.62
	2	Saddle Lake	Raw	Recreation area	20-Nov-00	0	0.241	<4	<4	N/A	3.8	7.92
	3	Water Treatment Plant	Raw	Raw & backwash	21-Nov-01	0	0.197	12	<4	N/A	2.8	7.34
	4	Stony Creek	Raw	D	21-Nov-01	0	0.082	4	<4	N/A	9.1	7.31
	5	Stony Creek	Raw	E	21-Nov-01	0	0	44	<4	N/A	38.0	8.12
	6	Stony Creek	Raw	F	21-Nov-01	0	0	4	<4	N/A	6.5	7.01
WINTER	7	Health Centre	Potable	Garage	30-Jan-01	ND*	0	0	0	<10	1.5	ND
	8	Water Treatment Plant	Potable		30-Jan-01	0	0	0	0	<10	0.3	ND
	9	Water Treatment Plant	Raw	Raw & backwash	30-Jan-01	0.129	0.088	<4	<4	N/A	1.6	ND
	10	Health Centre	Potable	Garage	22-Feb-01	0.972	0	0	0	<10	0.9	7.17
	11	Water Treatment Plant	Potable		22-Feb-01	0.041	0	0	0	<10	0.9	7.16
	12	Stony Creek	Raw	F	22-Feb-01	0	0	<4	<4	N/A	6.7	6.82
	13	Water Treatment Plant	Raw	Raw & backwash	22-Feb-01	0	0	<4	<4	N/A	1.6	6.54
SPRING	14	Health Centre	Potable	Garage	05-Apr-01	0	0	0	0	<10	1.0	7.23
	15	Water Treatment Plant	Potable		05-Apr-01	0.004	0	0	0	<10	0.4	7.17
	16	Water Treatment Plant	Raw	Raw & backwash	05-Apr-01	0	0	2**	0	N/A	2.1	7.76
	17	Saddle Lake	Raw	Below pumphouse	05-Apr-01	ND	0	0	0	N/A	4.0	7.99
	18	Stony Creek	Raw	F	05-Apr-01	0.222	0	0	0	310	7.0	7.94
	19	Health Centre	Potable	Garage	23-Apr-01	0	0	0	0	<10	0.4	7.21
	20	Water Treatment Plant	Potable		23-Apr-01	0	0	0	0		0.4	7.01
	21	Water Treatment Plant	Raw	Raw & backwash	23-Apr-01	0	0	8	<4	N/A	3.4	7.36
	22	Backwash Pond	Raw	Backwash recycle	23-Apr-01	0	0	8	<4	N/A	2.2	7.36
	23	Health Centre	Potable	Garage	07-May-01	0	0	0	0	180	0.2	7.6
	24	Water Treatment Plant	Potable		07-May-01	0	0	0	0	<10	0.2	7.3
	25	Vine Lagoon Effluent	Raw	Near Surface	07-May-01	0.484	0	>8000	5300	N/A	19.8	7.72
	26	Vine Lagoon Effluent	Raw	Near Bottom	07-May-01	ND	0	>8000	4800	N/A	16.6	7.56
	27	Stony Creek	Raw	F	07-May-01	ND	0	290	<4	N/A	6.4	8
	28	Water Treatment Plant	Raw	Raw & backwash	07-May-01	0	0	16	<4	N/A	3.1	8.35
SUMMER	29	Health Centre	Potable	Garage	16-Jul-01	0	1.623	Samples received without coolant. Results may not be representative of the quality of the water when it was collected			0.2	7.46
	30	Water Treatment Plant	Potable		16-Jul-01	0	0				0.2	7.44
	31	Water Treatment Plant	Raw	Raw & backwash	16-Jul-01	0	0				4.2	9.13
	32	Stony Creek	Raw	F	16-Jul-01	0	0				4.2	ND
	33	Health Centre	Potable	Boiler room (See37)	03-Aug-01	0	see 37				0.5	7.7
	34	Water Reservoir	Potable	In townsite	03-Aug-01	0	0.004				0.4	7.6
	35	Water Treatment Plant	Potable		03-Aug-01	0	0.026				0.2	7.58
	36	Water Treatment Plant	Raw	Raw & backwash	03-Aug-01	0	0.041				4.4	9.16
	37	Health Centre	Potable	Re-sampled 33	15-Aug-01	0	0.004				0.2	7.9

5.5 DISCUSSION

All potable water samples appear to comply with Canadian Drinking Water Guidelines for the parameters of total and faecal coliforms, heterotrophic plate counts, turbidity (except for one sample) and pH (Health Canada 1999). No total or faecal coliforms were detected and less than 10 CFU/mL in HPCs were detected in all samples except for one which reported 180 CFU/mL, which is acceptable according to Health Canada (1999). pH ranged between 6.5 and 8.5 in all potable water samples and only one sample, sample 7, exceeded the maximum allowable concentration of 1 NTU. There are currently no Canadian guidelines for *Giardia* spp. and *Cryptosporidium* spp. concentrations in potable water.

As per Canadian recreational water quality (Health and Welfare Canada 1992) the coliform data cannot be manipulated to test against Canadian regulations.[21] All but two samples gave rise to pH values between 6.5 and 8.5 (CCME 1999).

Inconsistent data presentations and data analyses plague protozoan detection studies, making them greatly difficult to compare. Due in part to data that falls within lower detection limits, geometric, arithmetic or no means at all are calculated at the author's discretion, often creating a biased presentation of data. The comparison of studies is further complicated by inconsistent protozoan recoveries and variances created by numerous methodologies. Furthermore, studies vary greatly in size and scope. Therefore the comparisons outlined in Table 15 is intended to be a broad comparison and illustrates examples of other studies while each cited study should be examined individually for a more meaningful comparison. Detection data obtained from this study was not compiled into geometric means due to the large proportion of values, that fall below the detection limit (reported as zero) that would require censoring.

A comparison of values within Table 15 reveals that Saddle Lake water treatment plant intake water contained average to low numbers of (oo)cysts while the recoveries (performed on water treatment plant intake waters) are comparable with, if not higher, than other studies.

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[21] Current Guidelines for Canadian Recreational Water Quality state that assuming 90 percent of the faecal coliforms are *E. coli*, the geometric mean of al. least 5 samples, taken during a period not to exceed 30 days, should not exceed 200 faecal coliforms/ 100 ml.

• Table 15: Comparison of surface water protozoa detection studies (WTP denotes water treatment plant).

Source water	*Cryptosporidium* spp. oocysts	*Giardia* spp. cysts	Recovery efficiencies	Detection method	Study
United States 17 States *(Surface waters)*	55% Positive Geom. mean: 43 oocysts/ 100L	16% Positive Geom. mean: 3 cysts/100L	29-58% *Cryptosporidium* 13-22% *Giardia*	Polypropylene yarn-wound filters, elution, sonication, IFA	(Rose et al. 1991)
Canada 72 municipalities *(Raw surface water)*	4.5% Positive	21% Positive	0-5% *Cryptosporidium* 2-47% *Giardia*	Polypropylene yarn-wound filters, manual elution, FITC staining, microscopy	(Wallis et al. 1996)
Ottawa, Canada Britannia WTP Lemieux WTP *(WTP sources)*	50% Positive Arith. mean: 4 oocysts/ 100L 100% Positive Arith. mean: 22 oocysts/ 100L	83% Positive Arith. mean: 6 cysts/ 100L 67% Positive Arith. mean: 6 cysts/ 100L	Ottawa river recoveries: 25.9±15.1% *Cryptosporidium* 13.3±5.5 *Giardia* Rideau river recoveries: 15.5±5.7% *Cryptosporidium* 5.5±2.4% *Giardia*	Standard methods 18th Ed.	(Chauret et al. 1995)
Alberta, Canada Rocky Mountain House Devon *(WTP sources)*		Geom. mean: 55.0 cysts/ 100L Geom. mean: 113 cysts/ 100L	Estimated recoveries: 42% *Cryptosporidium* 48% *Giardia*	ICR	(Shepel 2000)
Alberta, Canada Saddle Lake *(WTP source)*	37.5% Positive	12% Positive	20±12% *Cryptosporidium* 45±18% *Giardia*	Filta-Max, FITC staining, IMS, FACS	Present study

As indicated by the previous segment on recovery trials, (oo)cyst detection recoveries are likely variable in part due to varying water matrices. To determine the actual recovery of (oo)cysts it would be desirable to determine percent recoveries and variability with the identical water matrices to the one that was being tested. Without this information it is difficult to extrapolate the actual number of (oo)cysts present in natural and treated water. Also, the inconsistent volumes analyzed by FACS increased the variability of recoveries. Initial data files were conducted so that plots could be produced to show the distribution of particles to the gates, should they be required for trouble-shooting. In hind-sight it would have been preferable to sort the entire sample to obtain more accurate recoveries of (oo)cysts.

The health risks of drinking Saddle Lake potable water appear to be low when data were compared with health risk studies. Haas and Rose (1995) propose and action level of 10 to 30 oocysts per 100 L; only one *Cryptosporidium* spp. sample from potable water (sample 29) would indicate a need for re-sampling. Only one sample, sample 29, was above this level although the water sample did not appear to be infectious to mice.

The risk of obtaining giardiasis from Saddle Lake treated water also appears to be low. Rose and Gerba (1991) propose that cysts should be below 0.2 cysts/100 L while Wallis et al. (1996) propose an action level of 3 to 5 cysts/ 100 L in potable water. Using either of these levels, no *Giardia* spp. samples indicate human health risks. However, it should be noted again that two samples created giardiasis infections in gerbils, indicating a possible health risk.

This study examined treated water that had been treated with chlorine. The detection technique used in this study did not employ sodium thiosulfate as a means of neutralizing the chlorine residual remaining within the treated water. Although controversial, chlorine residuals may not affect (oo)cysts. Payment (1999) found that the more resistant microorganisms such as sporulated bacteria and viruses were unaffected by total residual chlorine concentrations of up to 0.9 mg/L and free chlorine concentrations of up to 0.7 mg/L over a 24 hour period. This may suggest that the residual chlorine concentrations found in the Saddle Lake distribution system may not affect *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts. For comparison, Saddle Lake water treatment plant attempts to maintain concentrations of 0.4 and 1.3 to 1.4 mg/L of free and total chlorine concentrations, respectively, although they can be as high as 1.23 and 2.20 mg/L, respectively[22].

Due to the small number of samples, the variability in protozoa presence and the variability in the detection method, there does not appear to be a trend in occurrence within the drainage basin. Future sampling should incorporate a larger portion of drainage basin with a greater number of samples. Samples should also be entirely sorted with FACS and the detection technique should be examined as noted in the previous recovery trial section.

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[22] As per a conversation with a water treatment plant operator at Saddle Lake.

Chapter 6

6 SADDLE LAKE SURVEY

6.1 INTRODUCTION

Residents of Saddle Lake First Nations Reserve responded to surveys conducted by the community that were aimed at determining land-use characteristics within their portion of the drainage basin. Results were utilized to determine if a) any relationships existed between land use and protozoa detections and b) if any land use or land management practices posed a significant threat to the drinking water source, Saddle Lake. The following is an overview of the results compiled using only those inhabitants within the drainage basin.

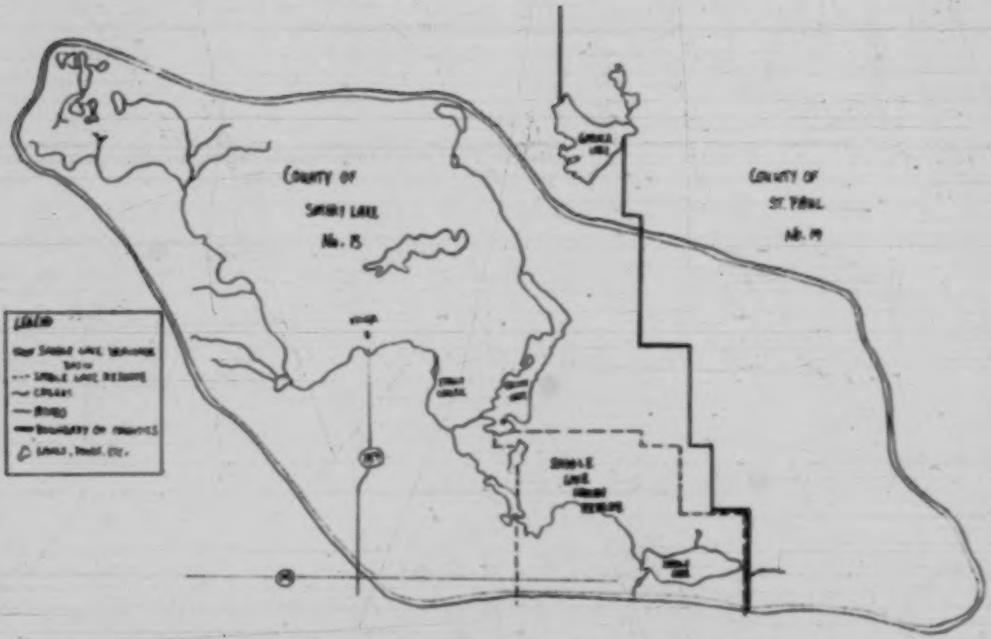
6.2 BACKGROUND

6.2.1 Drainage Basin and Source Water

Saddle Lake is the drinking water source for the majority of the Saddle Lake community. The lake's drainage basin consists of flat to undulating prairie land with variable amounts of agriculture with an area of approximately 704 km^2 (Figure 6). The northern portion of Saddle Lake Indian Reserve that is part of the drainage basin comprises approximately 10 percent of the drainage basin. The remainder of the drainage basin is comprised of portions of the Counties of St. Paul and Smoky Lake, which encompass 24 and 66 percent of the drainage basin, respectively.

6.3 EXPERIMENTAL DESIGN

See Appendix A for Health Canada's "Land-Use, Water and Waste Survey Form." Results were compiled by the community and are based on surveys from respondents residing within the drainage basin on Saddle Lake Reserve (Figure 6, within dashed perimeter). Only results from the land-use and wastewater treatment portions are summarized in the following section.


LEGEND
SADDLE LAKE DRAINAGE BASIN
SADDLE LAKE RESERVE
CREEKS
ROADS
BOUNDARY OF COUNTIES
LAKES, PONDS, ETC.
COUNTY OF
SMOKY LAKE
No. 13
COUNTY OF
ST. PAUL
No. 19
STONY CREEK
SADDLE LAKE INDIAN RESERVE

• Figure 6: The Saddle Lake drainage basin as inferred by contour maps and maps adapted from Land Data Technologies Ltd. of the Counties of Smoky Lake and St. Paul (1997, 2000).

6.4 RESULTS AND DISCUSSION

Note that individual properties on the reserve are not well defined. As such many land characteristics have not been included in survey results, as residents have not indicated that it was on their property. The following results are only from those dwellings that are occupied, of which all single-family dwellings with the exception of a few mobile homes.

Due to the low number of water samples drawn from Stony Creek and Saddle Lake, within the drainage basin, it would be difficult to correlate the following information with high or low numbers of protozoa. Therefore the following is intended more as a summary of the land use and sewage disposal information obtained within the drainage basin based on survey results.

Note again that the Saddle Lake drainage basin encompasses an area of approximately 704 km^2 of which 10 percent is comprised of the northern portion of Saddle Lake Indian Reserve. Therefore approximately ninety percent of the area within the drainage basin was not assessed by survey (Figure 6).

6.4.1 Sewage and Potable Water

The Saddle Lake Indian Reserve residents within the Saddle Lake drainage basin reported no outhouses, package treatment plants, private lagoons, and subsurface/ disposal chambers. All respondents reported septic tanks as their sewage disposal system.

Of the 157 respondents, 130 houses had septic tanks greater than 9 m from their drinking water source, 13 houses had septic tanks within 9 m of their drinking water source, two houses were not applicable, and 12 had unknown distances. Many septic tanks were drained via shootouts and the distances from shootouts to drinking water sources were often not recorded. Very few distances from sewage disposal systems to water bodies were reported.

Drinking water sources were: 118 cisterns, 2-bottled water, 26-community system, 8 wells, and 3 were unknown. Contamination of cisterns may be of importance should the coverings be in disrepair. Also, should cisterns be located on a flood plain, as were 13 of the 156 cisterns in correctly documented surveys, their chance of contamination would be increased through inadequate coverings.

6.4.2 Land Use

The Saddle Lake drainage basin covers just over 700 km^2, of which approximately 70 km^2 is comprised of Saddle Lake First Nation Reserve. Within the drainage basin portion of the reserve, residents accounted for only 9 km^2. The small fraction of land accounted for in the survey is due in part to the lack of property lines on the reserve. It is therefore likely that the survey results overestimate the amount of lawn by not accounting for many of the natural areas, roads and water bodies. A summary of the land use results is compiled in Appendix B.

On the Saddle Lake First Nation's Reserve, and in the Saddle Lake drainage basin no agriculture, industry, feedlots and rivers were reported. Thirty-nine cows, 20 calves, 33 horses, 3 colts, 125 dogs, and 25 outdoor cats reside within the drainage basin portion of the reserve. No surveys indicated manure storage before applying to land. Only one respondent reported pesticide use in an unidentified quantity.

The land-use portion of the survey covered a total of 2142 acres[23] which was comprised of:

- 69 percent lawn,
- 26 percent treed,
- 2 percent pasture,
- 1 percent gravel, and tire track or path roads, and dwellings, each, and
- Less than 1 percent paved roads, waterfront, pond and marsh, each (Appendix B).

It was presumed that due to the low numbers of livestock residing within the area surveyed that slope and aspect were not important determinants of potential fecal matter runoff. Also, only 20 out of 152 of the correctly documented surveys responded with slopes of steep or moderate nature. Of these properties few had aspects facing north, northeast or northwest, which would have indicated slower evaporation thus increasing the probability of faecal contamination in water bodies, and fewer still reported livestock on these lands. All other surveys reported either on rolling hills or flat land.

The average number of bedrooms and bathrooms per dwelling was 3.0 and 1.3, respectively. The average number of tenants within each home was between five and six but they did occasionally exceed 11 people.

6.4.3 The Counties of Smoky Lake and St. Paul

The Saddle Lake drainage basin is much larger than the portion contained on the Saddle Lake reserve. It is presumed that approximately ninety percent of it is within the two adjacent counties. A portion of Smoky Lake County No. 13 comprises approximately 66 percent of the basin while a fraction of St. Paul County No. 19 entails about 24 percent of the area. See Table 15 for a breakdown of the farmland area classifications of the counties of St. Paul and Smoky Lake.

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[23] This total land area figure and the subsequent percentages are derived only from complete and correctly filled in land-use segments of the surveys.

- Table 16: Summary of farmland area classifications by land use adapted from Alberta Food and Rural Development (1996). All values are in acres.

County	Total Area of Farms	Land in Crops	Summerfallow[24]	Tame/ Seeded Pasture[25]	Natural Land for Pasture[26]	All Other Lands[27]
St. Paul	771,269	305,983	24,777	115,306	262,155	63,048
Smoky Lake	497,598	209,961	19,082	49,485	176,563	42,507

The two counties contained a large number of livestock (Table 15). As of 1996, St. Paul contained 90,851 cattle of which 29,088 are calves under one year. Smoky Lake had 43,038 cattle of which 16,029 were calves under one year. Both St. Paul and Smoky Lake had pigs in numbers of 29,847 and 8,865, respectively. St. Paul had a total of 2,282 sheep and lambs whereas Smoky Lake had a total of 1,665 total sheep and lambs. As of 1996 the counties had a total of 2,259 and 1,020 horses and ponies combined in St. Paul and Smoky Lake, respectively (Alberta Food and Rural Development 1996).

Thus the counties surrounding Saddle Lake First Nation Reserve, St. Paul and Smoky Lake, likely contribute more protozoa to the watershed due to larger numbers of livestock.

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[24] Summerfallow "includes the idle land that has not been worked or sprayed for more than one year" (Alberta Food and Rural Development 1996).

[25] Tame/ seeded pasture "refers to land that has been cultivated and seeded, or drained, irrigated, fertilized or controlled for brush or weeds; does not include areas to be harvested for hay, silage or seed" (Alberta Food and Rural Development 1996).

[26] Natural land for pasture "refers to native pasture, native hay, rangeland, grazable bush, etc." (Alberta Food and Rural Development 1996).

[27] All other lands includes "land on which farm buildings, barnyards, lanes, home gardens, greenhouses and mushroom houses are located; idle land; woodlots; sugarbush; tree windbreaks; bogs; marshes; sloughs; woodland; wetland; etc" and Christmas tree areas (Alberta Food and Rural Development 1996).

Chapter 7

7 PROJECT SUMMARY

7.1 CONCLUSION

Giardia spp. and *Cryptosporidium* spp. are present within the Saddle Lake drainage basin, in the Saddle Lake water treatment plant intake and within treated water. Three of 16 potable water samples were positive for *Giardia* spp. Four of 16 potable water samples were positive for *Cryptosporidium* spp. In raw water samples, 3/17 and 5/20 samples were positive for *Giardia* spp. and *Cryptosporidium* spp., respectively while 1/8 and 3/8 water treatment plant raw water were positive for the protozoa. No samples induced cryptosporidiosis in mice while two potable water samples gave rise to giardiasis in gerbils. The town of Vilna's lagoon and Stony Creek did not appear to contribute large numbers of (oo)cysts to Saddle Lake at the time of sampling.

Due perhaps to the low numbers of samples and to the variability of the detection method, no correlations were found between the presence of (oo)cysts and total and faecal coliform levels, heterotrophic plate counts, turbidity and pH.

Surveys indicate that low numbers of livestock and modest amounts of agriculture exist on the reserve in contrast to the surrounding areas; it is likely that the counties surrounding Saddle Lake Indian Reserve contribute more contaminants, such as protozoa, to the drainage basin.

Recovery trials indicate that the current techniques need to be further optimized as the methods give rise to low percent recoveries with a large degree of variation as given by mean *Giardia* spp. and *Cryptosporidium* spp. percent recoveries of 44.8 and 19.9 percent and coefficients of variation of 37 and 59 percent, respectively.

7.2 RECOMMENDATIONS

- As per Table 7, further optimization of the current detection method is recommended with emphasis on seeding preparation, IMS optimization or exclusion, antibody optimization by pellet size, and staining procedures.
- Animal infectivity should be used as a qualitative measure of infectivity or only after large numbers of (oo)cysts have been detected in source waters due to the high ID_{50}'s for mice and gerbils.
- Additional sampling must be performed to detect trends in a drainage basin study. To approximate actual recoveries, matrix spike samples should be performed on all source waters when performing detection assays.
- To determine land use characteristics within a drainage basin, surveys should be distributed to all residents within the basin and should include questions relating to riparian buffer strips bordering water bodies. Aerial photos would also facilitate land-use characterization, especially within areas such as First Nation Reserves where property lines are not well defined.

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Comprehensive Drainage Basin Study of Potential Micro-Organism Contamination of the Saddle Lake Water Supply

LANDUSE, WATER AND WASTE SURVEY FORM

This survey form was prepared in partnership with the Saddle Lake First Nation, Indian and Northern Affairs Canada and Health Canada

9.1 A. DWELLING INFORMATION

First Nation Name: Saddle Lake

Community Name: Saddle Lake

Area or Subdivision Name: ____________________

Dwelling Owner/ Tenant's Name: ____________________

Road Number: ____________________

Dwelling Number: ____________________

Type of Dwelling: ☐ Single Family Dwelling ☐ Mobile Home/ Trailer

☐ Duplex/ Triplex/ Fourplex ☐ Other

Year Dwelling was constructed: ____________________

Legal Land Description of Property: ____________________

Is the Dwelling occupied? ☐ Yes ☐ No (Vacant/ Abandoned)

Length of Occupancy: ____________________

If length of occupancy is less than 5 years, previous owners/ occupants in past 5 years:

____________________ ____________________

Health Canada's Site Code: ____________________ ☐ Not listed

9.2 B. LAND-USE AND DESCRIPTION

1. What is the size of your property (in hectares, acres or meters)? ____________________

2. Estimate the **percent** of your property that is:
   - ☐ treed: __________________
   - ☐ lawn (primarily grass): ____________________
   - ☐ dwellings: _______________
   - ☐ road:
   - ☐ paved: _________________
   - ☐ gravel: _________________
   - ☐ tire tracks or paths: ____________________
   - ☐ waterfront (beach or rocky area bordering water): _______
   - ☐ stream or river: _________________
   - ☐ pond: __________________
   - ☐ marsh: _________________
   - ☐ feedlot : _____________
   - ☐ pasture: ________________
   - ☐ agriculture: ______________
   - ☐ industry: ________________ what kind of industry? ___
   - ☐ other: __________________ percent: _____________
   - ☐ other: __________________ percent: _____________

3. Would you consider your property to be on:
   - ☐ a steep slope
   - ☐ a moderate slope
   - ☐ rolling hills
   - ☐ flat land

4. If you have chosen either **steep or moderate slope** in the previous question, in approximately what direction does your land face?
   - ☐ North
   - ☐ North-East
   - ☐ East
   - ☐ South-East
   - ☐ South
   - ☐ South-West
   - ☐ West
   - ☐ North-West

5. Is the dwelling or land located in a flood plain?
   - ☐ Yes
   - ☐ No

6. Is the dwelling or land located in a high water table area?
   - ☐ Yes
   - ☐ No

7. How many bedrooms are in the dwelling?
    - ☐ 1
    - ☐ 2
    - ☐ 3
    - ☐ 4
    - ☐ 5
    - ☐ 6 or more

8. How many bathrooms are in the dwelling?
    - ☐ 1
    - ☐ 2
    - ☐ 3 or more
    - ☐ does not have a bathroom

9. How many people live in the dwelling (on average if it fluctuates)?
    - ☐ 1-3
    - ☐ 4-5
    - ☐ 6-7
    - ☐ 8-10
    - ☐ 11 or more

10. Complete the following table with the types and numbers of pets that reside on or spend significant amounts of time on your property. Please exclude indoor pets.

Type of Animal	Number
dogs	
cats	

11. Complete the following table with the types and corresponding numbers of livestock that reside or spend significant amounts of time on your property.

Type of Animal	Number
cows: adult	
calves	
horses: adult	
colts	

12. Indicate if any of the following are applied on your property and the respective amounts utilized:

- ☐ pesticides: if yes, how much per year? ____________________
- ☐ herbicides: if yes, how much per year? ____________________
- ☐ fertilisers:
  - ☐ manure: if yes, how much per year? ____________________
  - ☐ other ________________: how much per year? ________________

13. If you spread manure on your land approximately how many days do you store it before spreading it on your fields? Where and how do you store it?

____

9.3 C. DRINKING WATER

1. Do the occupants drink the water servicing the dwelling?
   - ☐ Yes, with no further treatment **(proceed to section D)**
   - ☐ Yes, but only after further treatment **(proceed to questions 4 and 5)**
   - ☐ No **(proceed to questions 2 and 3)**

2. If NO, why?
   - ☐ Taste
   - ☐ odour
   - ☐ colour
   - ☐ high turbidity or cloudiness
   - ☐ Other

3. If NO, from where do the occupants get their drinking water?
   - ☐ Bottled water
   - ☐ Water from another dwelling: who's dwelling? ____________________
   - ☐ Community membrane system
   - ☐ Other: ____

4. If YES, is the water treatment apparatus working?
   - ☐ Yes
   - ☐ No

5. What type(s) of water treatment unit(s) are utilised?
   - ☐ Distillation
   - ☐ Water softener
   - ☐ Reverse osmosis
   - ☐ Iron removal
   - ☐ Not sure what it is
   - ☐ Other ____

9.4 D. WATER SUPPLY

1. The water source for the dwelling is a:
   - ☐ Private Well ☐ Cistern ☐ Other
   - ☐ Spring ☐ Community System
   - ☐ Barrel ☐ Dugout

If dwelling water supply is Community based, proceed to section E (Sewage).

9.4.1 PRIVATE WATER SUPPLY ONLY (Questions 2-4)

2. Is the private water supply located in a flood plain?
   - ☐ Yes ☐ No

3. How many homes does this private water supply serve?
   - ☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6 or more

   If more than 1, please list other dwellings served by this water supply:

   ____

   ____

   ____

4. On which side of the dwelling is the private water supply located?
   - ☐ North ☐ South ☐ East ☐ West
   - ☐ Basement ☐ Not Applicable

If dwelling water supply is a cistern or barrel, please proceed to questions 5 to 12.
If dwelling water supply is a well, please proceed to questions 13 to 15.

9.4.2 CISTERNS AND BARRELS ONLY (Questions 5 to 12)

5. The cistern/ barrel is located:
   - ☐ In the dwelling in the basement ☐ In the dwelling on the main floor
   - ☐ Outside in a low lying area ☐ Outside in a level area
   - ☐ Outside in an elevated area ☐ Other
   - ☐ Outside in above ground in shed
   - ☐ Water not supplied by barrel/ cistern

6. How old is the well / cistern?
   - ☐ 0-5 years ☐ 5-10 years ☐ 10-15 years
   - ☐ 15-20 years ☐ 20 years or more

7. The access/ manhole opening to the cistern is:
   - ☐ Above ground level (______ mm or ______ inches above ground)
   - ☐ Below ground level (Buried)
   - ☐ At ground level
   - ☐ Water not supplied by cistern

8. The condition of cistern access/ manhole opening cover is:
   - ☐ Satisfactory (intact and tight-fitting)
   - ☐ Missing
   - ☐ Improper Lid (not tight-fitting, wooden, etc)
   - ☐ Damaged
   - ☐ Water not supplied by cistern

9. Is the cistern lid level?
   ☐ Yes ☐ No ☐ Water not supplied by cistern

10. The ground around the access/ manhole opening is:
    - ☐ Sloping away from the cistern opening
    - ☐ Level with the cistern opening
    - ☐ Sloping towards the cistern opening
    - ☐ Water not supplied by cistern

11. Condition of the access/ manhole opening extension:
    - ☐ Satisfactory with water-tight joints
    - ☐ Not properly sealed
    - ☐ Water not supplied by cistern
    - ☐ Cracked/ damaged
    - ☐ Other

12. The ground around the cistern access/ manhole opening is:
    - ☐ Loose
    - ☐ Compacted
    - ☐ Water not supplied by cistern

9.4.3 WELLS ONLY (Questions 13 to 15)

13. The well is located:
    - ☐ In the basement of the dwelling
    - ☐ Outside in a low lying area
    - ☐ Outside in a level area
    - ☐ Outside in an elevated area
    - ☐ Other ______________

14. The ground around the well casing is:
- ☐ Sloping away from well
- ☐ Level around the well
- ☐ Sloping towards the well
- ☐ Water not supplied by well

15. The ground around the well casing is:
- ☐ Loose
- ☐ Compacted

9.5 E. SEWAGE

1. Is the dwelling connected to a community sewage system?
   ☐ Yes ☐ No
   If yes which community system? ______________________________

If dwelling is connected to a community sewage system, proceed to section F (Solid Waste Disposal Section).

2. To which type of private disposal system is the dwelling connected?
   - ☐ holding tank or septic tank **(proceed to questions 3 to 15)**
   - ☐ subsurface disposal field or chamber field **(proceed to questions 16 to 21)**
   - ☐ open discharge **(proceed to questions 22 to 33)**
   - ☐ private lagoon only **(proceed to questions 34 to 41)**
   - ☐ package treatment plant
   - ☐ outhouse **(proceed to questions 42)**
   - ☐ other

9.5.1 SEPTIC TANK/ HOLDING TANK

3. Is the septic tank/holding tank, including the access/ manhole opening completely buried?
   - ☐ Yes
   - ☐ No

4. Tank material:
   ☐ Concrete ☐ Fibreglass ☐ Plastic
   ☐ Polyethylene ☐ Steel

5. Type of septic tank/holding tank:
   ☐ One chamber ☐ Two chambers
   ☐ 2 - One chamber Tanks ☐ Other

6. The access/ manhole opening is:
   - ☐ Above ground level (______mm or ______ inches above ground)
   - ☐ Below ground level (buried)
   - ☐ At ground level

7. Condition of access/ manhole opening cover:
   - ☐ Satisfactory (intact and tight-fitting)
   - ☐ Missing
   - ☐ Improper Lid (not tight-fitting, wood, etc)
   - ☐ Damaged
   - ☐ Other: ____________________

8. Condition of the access/ manhole opening extension:

   ☐ Satisfactory ☐ Cracked/ damaged

   ☐ Not properly sealed ☐ Missing ☐ other : __________

9. The ground around the access/ manhole opening is:
   - ☐ Sloping away from the tank opening
   - ☐ Level with the tank opening
   - ☐ Sloping towards the tank opening

10. The ground around the access/ manhole opening is:

    ☐ Loose ☐ Compacted

11. Is there evidence of sewage overflowing or overflowed from the septic tank/holding tank?

    ☐ Yes ☐ No

12. The septic tank or holding tank is located:

    ☐ less than OR ☐ equal to or greater than
    9 m (30 ft) from a water course

    Which water course?

    ____

    ☐ less than OR ☐ equal to or greater than
    9 m (30 ft) from a drinking water source

    Which drinking water source?

    ____

13. How old is the septic or holding tank?

    ☐ 0-5 yrs ☐ 5-10 yrs ☐ 10-15 yrs

    ☐ 15-20 yrs ☐ 20 yrs or more

14. When was the septic tank last pumped out?
- ☐ Never
- ☐ More than 1 year
- ☐ Do not have a septic tank
- ☐ Less than 1 year
- ☐ Do not know

15. How often is your holding tank pumped out?
- ☐ Once per week
- ☐ More than 3 times per week
- ☐ Once in 3 weeks
- ☐ Do not have holding tank
- ☐ Twice per week
- ☐ Once in 2 weeks
- ☐ Once in a month

9.5.2 SUBSURFACE DISPOSAL FIELD/ CHAMBER FIELD

16. How many homes are serviced by this system?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more

If more than 1, please list other homes on system:
____
____
____

17. Where is the disposal field located?
- ☐ In a level area
- ☐ In a low lying area
- ☐ In an elevated area

18. What type of vegetation is growing on the disposal field?
☐ Grass ☐ Trees ☐ Dirt/Bare ☐ Shrubs
☐ Other ____________________________

19. What is located over the disposal field?
- ☐ Nothing
- ☐ Driveway
- ☐ Buildings
- ☐ Visible signs of vehicle traffic
- ☐ Livestock
- ☐ Parking area
- ☐ Other ________________

20. Are there any indications that the disposal field is malfunctioning (leaking sewage or temporary surface discharge)?
- ☐ Yes
- ☐ No

21. Is any part of the sewage disposal field uncovered?

- ☐ Yes
- ☐ No

9.5.3 OPEN DISCHARGE (Shoot-outs, ejectors, etc)

22. The location of the open discharge is:

- ☐ North
- ☐ North-East
- ☐ East
- ☐ South-East
- ☐ South
- ☐ South-West
- ☐ West
- ☐ North-West

23. Is the effluent line uncovered?
- ☐ Yes
- ☐ No

24. Where is the sewage discharge point located?
☐ In trees ☐ In tall/ thick vegetation ☐ In an open area
☐ In garden ☐ other ______________________________

25. Is the effluent discharge pipe damaged or broken?
- ☐ Yes
- ☐ No

26. Is the ground around the effluent discharge pipe mounded?
- ☐ Yes
- ☐ No

27. If YES, what is the height of the mound?
_______ mm OR _______ inches

28. If YES, what material is used in the mound?
☐ Field Stone ☐ Gravel ☐ Dirt
☐ Other_____________

29. Is the effluent discharge point located in a low-lying area?
- ☐ Yes
- ☐ No

30. Is effluent accumulating around the discharge area?
- ☐ Yes
- ☐ No

31. Does the sewage effluent flow towards a water course or source?
- ☐ Yes
- ☐ No

32. If YES, which one? _________________________

33. What is the distance from the dwelling to the discharge point? _____________m

9.5.4 PRIVATE LAGOON

34. How many homes are serviced by this system?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more

If more than 1, please list other homes on system:
____
____
____

35. Is the lagoon bermed?
☐ Yes ☐ No

36. If YES, is the berm vegetation maintained? (i.e., mowed)
☐ Yes ☐ No

37. Is there any indication of berm erosion?
☐ Yes ☐ No

38. Is effluent overflowing the lagoon berm?
☐ Yes ☐ No

39. Are there any indications of sewage seeping from the lagoon?
☐ Yes ☐ No

40. Does the lagoon have a discharge point?
☐ Yes ☐ No

41. If YES, is there sewage discharging from the lagoon on a continuous basis?
☐ Yes ☐ No

9.5.5 OUTHOUSE

42. What is the distance from the outhouse to a:

- water course? __________________ m
- drinking water source? __________________ m

9.5.6 ACTUAL DISTANCE SETBACK SUMMARY (in metres)

	First Sewage Disposal System (type: ________)	Second Sewage Disposal System – if applicable (type:____________)
Closest Drinking Water Supply (specify the type, e.g. well, spring, lake, etc.)		
Closest Water Body (specify the type, e.g. lake, river, stream)		
Second Closest Water Body (specify the type, e.g. lake, river, stream)		

9.6 F. SOLID WASTE DISPOSAL

1. How is the garbage stored outside the dwelling/trailer?
   - ☐ In metal containers with tight-fitting lids
   - ☐ In sealed garbage bags
   - ☐ In wooden containers with lids
   - ☐ It is not contained (evidence of littering)
   - ☐ Stored on wooden stand
   - ☐ Garbage not stored outside
   - ☐ Other

2. How is the garbage collected?
   - ☐ Picked up by garbage truck
   - ☐ Dropped off at the landfill site or transfer station by home owner
   - ☐ Burned on site
   - ☐ Disposed into a garbage pit on site

3. The garbage storage is located:

   ☐ less than OR ☐ equal to or greater than
   9 m (300 ft) from a water course

   ☐ less than OR ☐ equal to or greater than
   9 m (300 ft) from a drinking water source

4. Which water course? ______________________

5. Which drinking water source? ______________________

9.7 G. ADDITIONAL COMMENTS

9.8 H. DRAWING

Please draw a site plan that includes the following: dwelling/ trailer, sewage system, driveway, roads, property line (if known), manure storage area, pasture (feedlots, etc), water bodies (streams, rivers etc.) and drinking water source (e.g. well/cistern). In addition include anything else that is relevant to water quality issues.

COMPLETED BY: **DATE:**

As the dwelling owner/ tenant, I understand that this survey is to help identify and document various housing issues and may be used by the First Nation for future planning. This does not imply that the noted areas of concern may be repaired or that a new dwelling will be constructed for me.

Dwelling/ Trailer OWNER'S SIGNATURE:

Appendix B

10 LAND USE SURVEY RESULTS

- Table 17: Summary of land use data from Health Canada's "Landuse, Water and Waste Survey Form".

SIZE (acres)	PERCENT									
	Treed	Lawn	Dwellings	Road			Waterfront	Pond	Marsh	Pasture
				Paved	Gravel	Paths				
2	0	100	0	0	0	0	0	0	0	0
2	60	40	0	0	0	0	0	0	0	0
2	10	90	0	0	0	0	0	0	0	0
30	90	1	0	0	0	x	9	0	0	0
2	40	60	0	0	0	0	0	0	0	0
2	0	100	0	0	0	0	0	0	0	0
3	10	90	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	11	89	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	10	90	0	0	0	0	0	0	0	0
650	20	10	0	0	10	10	0	0	0	50
2	0	98	1	0	1	0	0	0	0	0
300	20	5	1	0	1	0	0	20	0	53
10	90	5	5	0	0	x	0	0	0	0
5	90	9	1	0	0	0	0	0	0	0
25	20	60	1	0	2	0	7	0	0	10
250	90	9	1	0	0	0	0	0	0	0
20	7	85	8	0	0	0	0	0	0	0
2	0	100	0	0	0	0	0	0	0	0
2	0	100	0	0	0	0	0	0	0	0
200	0	8	0	0	2	0	0	0	0	90
2	10	90	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
5	80	15	0.5	0	4.5	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
	0	100	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	10	90	0	0	0	0	0	0	0	0
4	0	60	2	0	38	0	0	0	0	0
2	0	100	0	0	0	0	0	0	0	0

SIZE (acres)	PERCENT									
	Treed	Lawn	Dwellings	Road			Waterfront	Pond	Marsh	Pasture
				Paved	Gravel	Paths				
300	25	75	0	0	0	0	0	0	0	0
2	40	60	0	0	0	0	0	0	0	0
2	10	90	0	0	0	0	0	0	0	0
1	0	89	1	0	10	0	0	0	0	0
10	45	36	9	10	0	0	0	0	0	0
50	80	18	1	0	1	0	0	0	0	0
1.5	0	90	10	0	0	0	0	0	0	0
1.5	5	90	5	0	0	0	0	0	0	0
5	70	10	0	0	1	0	0	0	0	19
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	36	36	0	0	14	14	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	40	60	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	50	50	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0

SIZE (acres)	PERCENT									
	Treed	Lawn	Dwellings	Road			Waterfront	Pond	Marsh	Pasture
				Paved	Gravel	Paths				
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	52	34	0	0	7	7	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	25	75	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
3	10	90	0	0	0	0	0	0	0	0
1.5	99	0	1	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	0	100	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
20	87	10	3	0	0	0	0	0	0	0
4	0	90	2	0	8	0	0	0	0	0
5	0	95	1	0	4	0	0	0	0	0
2	25	75	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	0	86	3	0	11	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	70	30	0	0	0	0	0	0	0	0
5	20	40	0	0	6	0	0	0	17	17
2	0	90	2	0	8	0	0	0	0	0
1.5	0	80	10	0	0	10	0	0	0	0
2	60	40	0	0	0	0	0	0	0	0
2	53	25	0	0	11	11	0	0	0	0

SIZE (acres)	PERCENT									
	Treed	Lawn	Dwellings	Road			Waterfront	Pond	Marsh	Pasture
				Paved	Gravel	Paths				
2	11	84	5	0	0	0	0	0	0	0
2	42	21	21	0	8	8	0	0	0	0
2	10	90	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	36	36	0	0	14	14	0	0	0	0
3	5	70	2	0	23	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
5	5	90	0	0	1	0	4	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	40	60	0	0	0	0	0	0	0	0
2	79	0	0	0	10.5	10.5	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	40	60	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
2	30	70	0	0	0	0	0	0	0	0
3	40	60	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
2	20	80	0	0	0	0	0	0	0	0
MEAN	**26**	**69**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**2**
ACRES	**560**	**1478**	**15**	**2**	**30**	**13**	**3**	**3**	**3**	**37**